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TEXT-BOOK
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VOL. I
INVERTEBRATA

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TO

THE MEMORY OF

ADAM SEDGWICK

BELOVED TEACHER AND FAITHFUL FRIEND

THIS VOLUME

IS DEDICATED BY THE

AUTHOR

PREFATORY NOTE

The design of this Text-book of Embryology, of which this is the first volume, is to associate the structural development of embryos with broad generalizations of what is known of their physiology. Attention will be drawn, for instance, to the correlation between the function of certain organs of a larva and its habit of life, and, in a more general way, between function and habit and the course of development. Reference will be made to some of the more striking results obtained by Experimental Embryological research. Attention will be drawn to gaps in our knowledge which indicate promising fields for research.

It is hoped that the interest of all students of Embryology will thus be stimulated, and the practical value of these volumes, especially for students of medicine, ensured.

A second volume, by Professor Graham Kerr, in which the lower Vertebrata will be dealt with, will follow as soon as possible, and a third volume by Mr. Richard Assheton, on Mammals, will complete the work.

The Authors are responsible for the facts and generalizations recorded, and to them is due all the credit which may be given to the work.

THE EDITOR.

PREFACE

THIS book has been written in order to achieve two objects, first to place before the reader in as succinct a form as possible the best ascertained results in the field of Invertebrate Embryology, and secondly to indicate some of the problems which as yet remain unsolved and the best means of attacking them.

In order to attain the first object a number of typical life-histories, illustrating all the important groups of Invertebrata, have been described, and in selecting the types for special description two principles have guided us: first, the life-history of the type chosen must be thoroughly ascertained, and second, the type must be a common form easily accessible to students in temperate regions. Thus the spider has been chosen as a type of the Arachnida rather than the scorpion, and for the same reasons the life-histories of parasitic forms have been very slightly dealt with. The Trematoda and Cestoda have been entirely left out of consideration because it is difficult to obtain a complete series of the stages in the life-history of any one species—and though the external features of the life-history of members of these groups are known, their organogeny is still to be worked out. Moreover, the external features of the development of Trematoda and Cestoda are adequately described in ordinary text-books of zoology.

In pursuit of the second object the methods used by the best investigators have been given in connection with the description of the life-history of each type examined by them, and we have ever striven to keep before the mind of the student the idea that the ultimate object of the Science of Embryology is not solely the ascertaining of facts but especially the determination of the laws of life which underlie them; it is for these reasons we have endeavoured to make full use of the light which the new science of Experimental Embryology throws on these laws.

In a book of this compass, devoted to such an enormous subject as Invertebrate Embryology, very much must necessarily be omitted, but it seemed to us better to run the risk of criticism on this score and to bring our survey of the field to a conclusion within a reasonable period, rather than to attempt to give a complete account of all that is known of Invertebrate Embryology. Such an attempt would involve the task of writing not a single volume but a series of volumes; it would require for its accomplishment many years and would be beyond the powers of one man. Moreover, the first volume would be out of date long before the last volume was published.

The literature lists have been purposely kept as brief as possible—as a rule only the most recent papers on the subject have been cited. Where earlier papers have been referred to it is chiefly because these papers, in laying the foundation of our knowledge, have not been superseded by later work.

In conclusion, my best thanks are due to my colleagues, Professor Lefroy and Mr. Dobell, for valuable suggestions, and to my wife for much help in the tedious work of preparing the Index.

E. W. M.

Imperial College of Science, South Kensington,
July 29, 1914.

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ERRATA

Page 9, line 38, *for* "cell sap" *read* "cell and sap."
,, 61, lines 5 and 6, *for* "medusa *S. apicata*" *read* "medusa of *S. apicata*."
,, 125, line 22, *for* "first nucleus" *read* "nuclear matter of the sperm head."
,, 126, line 4, *for* "broken in two" *read* "broken into."

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CHAPTER I

INTRODUCTION

THE science of Embryology has for its subject-matter the growth of animals from the time when they first appear as germs in the bodies of their parents until they reach the adult condition, and are able to produce similar germs themselves. It thus includes a study of the complete life-cycle, and is much more extensive in its scope than ordinary descriptive Comparative Anatomy which confines itself to a study of the adult forms.

In practice, however, the study of the adult form precedes the study of all other stages of the life-history, because it is assumed that in the adult producing ripe germs, we have a stage which is the same whatever kind of animal we examine. A text-book of Embryology therefore assumes a knowledge of the adult forms of the animals whose life-histories it describes. Even with this limitation the scope of Embryology would be enormous were it not for a defect which is often overlooked, but which renders it possible to bring the most important results before the reader within moderate compass; this defect is the extreme difficulty of finding out with any completeness the whole course of any given life-history.

The life-history of an animal is only in the rarest cases directly observed; it is deduced from a comparison with one another of individuals of various ages, and only when we can examine a large number of individuals belonging to stages separated from one another by very short intervals can we get any reliable results. Numberless mistakes have been made in the past and will continue to be made in the future, by the effort to re-construct a life-history from the observation of an insufficient number of stages. Thus, to give examples, the germ cells of *Balanoglossus* and of the Annelid *Lopadorhynchus* have been stated to arise from the ectoderm, whereas in reality in both cases they arise from the lining of the body-cavity or coelom. Sometimes the life-history has been actually read backwards: thus the later stages in the Tornaria larva have been regarded as the earlier. In the case of the vast majority of animals only bits and scraps of the life-history are known, and the number of cases in which the whole course of this history is thoroughly known are very few. Hence a careful study of these few cases must suffice to include the certain and indisputable results of the science of embryology. Outside this limited field are a number of suggestive facts from which no certain conclusions can be drawn, but which serve as clues to indicate those lines for future research which will probably give the most interesting results; some of these will be referred to in the following pages.

The germs from which animals spring are of two kinds—(*a*) those that can develop directly, or **asexual** germs; and (*b*) those that under ordinary circumstances are incapable of development until they have united or "conjugated" with another germ. These latter are the **sexual** germs, and in all the animals which we shall have to consider they are unicellular, and are of two kinds, viz. comparatively large non-motile germs or **ova** (eggs), and small motile germs or **spermatozoa.** The organism which produces the ova is termed the **female,** that producing the spermatozoa, the **male,** and that which results from the union or **conjugation** of these two types of germ is called a **zygote.** If, as often happens, both kinds of germs are produced by the same adult, this is termed a **hermaphrodite.** In this case, however, the spermatozoa usually ripen before the ova and the animal is said to be **protandrous.** A case where the eggs ripen before the male germs is very rare amongst animals, but is commoner amongst flowering plants, such an organism being termed **protogynous.**

Most asexual germs are multicellular, containing several nuclei and in some cases portions of more than one tissue of the parent. Such germs are called **buds,** and the laws of bud development are as yet most imperfectly known, but in many cases they seem to differ markedly from those governing the development of the zygote. In this book our attention will be directed mainly to the development of the zygote, but the most important facts about bud development will also be given. Some asexual germs are called **parthenogenetic ova,** because in development and appearance they resemble true ova, from which they differ only in being able to undergo complete development without conjugating with spermatozoa. The development in this case is the same as that exhibited by true ova.

The development of ova and of spermatozoa, from their first appearance until they become capable of union with one another, is known as **gametogenesis,** since **gamete** is a convenient word to designate both kinds of sexual germ. It is called **oogenesis** for the ova and **spermatogenesis** in the case of spermatozoa.

Both forms of germ when first distinguishable are small, rapidly dividing cells which are often termed **primitive germ cells.** In these divisions the nuclear substance, which absorbs stain—the **chromatin**—becomes arranged in the form of a definite number of rods or **chromosomes**—denoted by the symbol $2x$. The actual number is characteristic of the species in question, and it is often assumed, and is taught in most text-books, that this number is characteristic of the nuclei of all the cells in the body when they proceed to division. But this is by no means universally the case; in fact in several cases which have been subjected to detailed examination it has been proved not to be the case.

The germ cells in this state of rapid division are termed **oogonia** and **spermatogonia** respectively. Division is succeeded in both cases by growth and then by a state of rest, at the end of which the germs are known respectively as **oocytes** and **spermatocytes of the first order.** In most cases during this period the difference between the two kinds of germ becomes apparent. The spermatocytes increase only slightly in size as compared with the spermatogonia and undergo no diminution in number; but some only of the oogonia increase in size and become the large oocytes or unripe ova; the remaining oogonia are either absorbed as food by their successful sisters, or reduced in size so as to form "**follicle cells**" which serve as a protective covering for the oocytes.

The follicle cells in many if not all cases contribute nourishment to the oocyte, and a considerable portion of this food, termed **deuteroplasm** or **food-yolk,** is precipitated in the form of globules or platelets consisting chiefly of **lecithin.** A first stage in the storing of food material in the cytoplasm appears to be the emission into the cytoplasm of chromatin from the nucleus. This process has been studied in detail in Echinoderm eggs by Schaxel (1911). Yolk globules appear later, at first near the periphery of the egg, by the transformation of this cytoplasmic chromatin. Often indeed the modified cytoplasm infiltrated with chromatin is aggregated in a more or less spherical body which absorbs stain, termed the **yolk-nucleus** (Fig. 11, *y.n*); round this the yolk first appears, but after the completion of yolk formation it disappears. Occasionally, as Schaxel has shown to be the case in the egg of *Strongylocentrotus,* no yolk at all is formed, the deposits of cytoplasmic chromatin constituting the only reserve material present. Different eggs differ from one another in the amount and nature of the food-yolk. The follicle cells in many cases have as their final duty the secretion of an outer **egg-shell** or **chorion** (Fig. 11, *ch*). The inner egg-shell is secreted after fertilization by the cytoplasm of the egg itself and is termed the **vitelline membrane.**

The nucleus of the unripe ovum is in nearly every case distinguished by the fact that it has the form of a vesicle containing a clear fluid termed the **nuclear sap,** within which is a dense mass of staining matter, different in chemical reactions from chromatin, which is termed the **nucleolus.** Stretched across the **nuclear sap** are a few fibres and on these the true chromatin is situated as small inconspicuous grains. This arrangement of material gives the nucleus of the oocyte a peculiar look which is unmistakable and which led to its being termed the **germinal vesicle** by the older writers, whilst the nucleolus was termed the **germinal spot.**

Before male and female germ cells can unite both must **mature,** and this they do by undergoing two **maturation divisions.** The changes which the nucleus undergoes previous to, and during, these maturation divisions, have been studied with great minuteness by a large number of observers, and on many important points a general agreement has been arrived at. An excellent summary of the present stage of our knowledge has been given by Agar (1911), who has worked out the development of the male cells in the Dipnoan fish *Lepidosiren*.

As maturation approaches, the chromatin granules in the nuclei of the spermatocytes of this species become aggregated into long ribbons, which are the chromosomes. This stage is termed **leptonema**, and the chromosomes in this stage are termed **leptotene** threads; they appear in double the number that are found in the ripe ovum when ready to receive the spermatozoon. This double number is known as 2 x. There is a large conspicuous nucleolus present as in the unripe egg (Fig. 1, A, n). The leptotene threads continue to shorten and become thicker and thus they pass into the stage of **zygonema**, in which the threads become opposed to one another in pairs and are termed **zygotene** threads (Fig. 1, B). Then the members of each pair fuse with one another. Thus the stage of **pachynema** is attained in which there are only x shorter thicker **pachytene** threads or chromosomes, arranged in the form of U's in a "bouquet" at one side of the nucleus.

Here we arrive at a fundamental divergence of opinion between two groups of workers. The changes which we describe cannot, of course, be observed in the living nucleus, but must be inferred from the comparison with one another of fixed and stained nuclei. Some workers maintain that nuclei with x pachytene threads represent the first stage in maturation, and that the stage with 2 x zygotene threads represents an attempt at longitudinal division of these chromosomes, which is, however, abortive. Agar, however, points out that in the material which he studied the leptotene threads cross each other at all angles, and when the process of amalgamation in pairs or **syndesis** begins, at first only one end of each member of a pair of zygotene threads is parallel with its fellow, the other end passes into an irregular tangle. He, therefore, in common with a great number of workers, interprets the appearances seen in the beginning of the pachytene stage as the beginning of a side-by-side fusion of originally separate chromosomes—*i.e.* as **parasyndesis**. This stage is followed by one in which the two elements forming the pachytene chromosome separate in the middle, and the chromosome is transformed into an elongated ring which is twisted on itself (Fig. 1, C). This is the stage of **strepsinema**, the rings being termed **strepsitene** threads. The two sides of each ring separate from one another, first at one end, so that the ring is converted into an elongated V, and then at the other so that the two original constituents of the ring are again entirely separated from one another and the original number of chromosomes, 2 x, is restored.

During the strepsinema stage a process called **synizesis** begins.

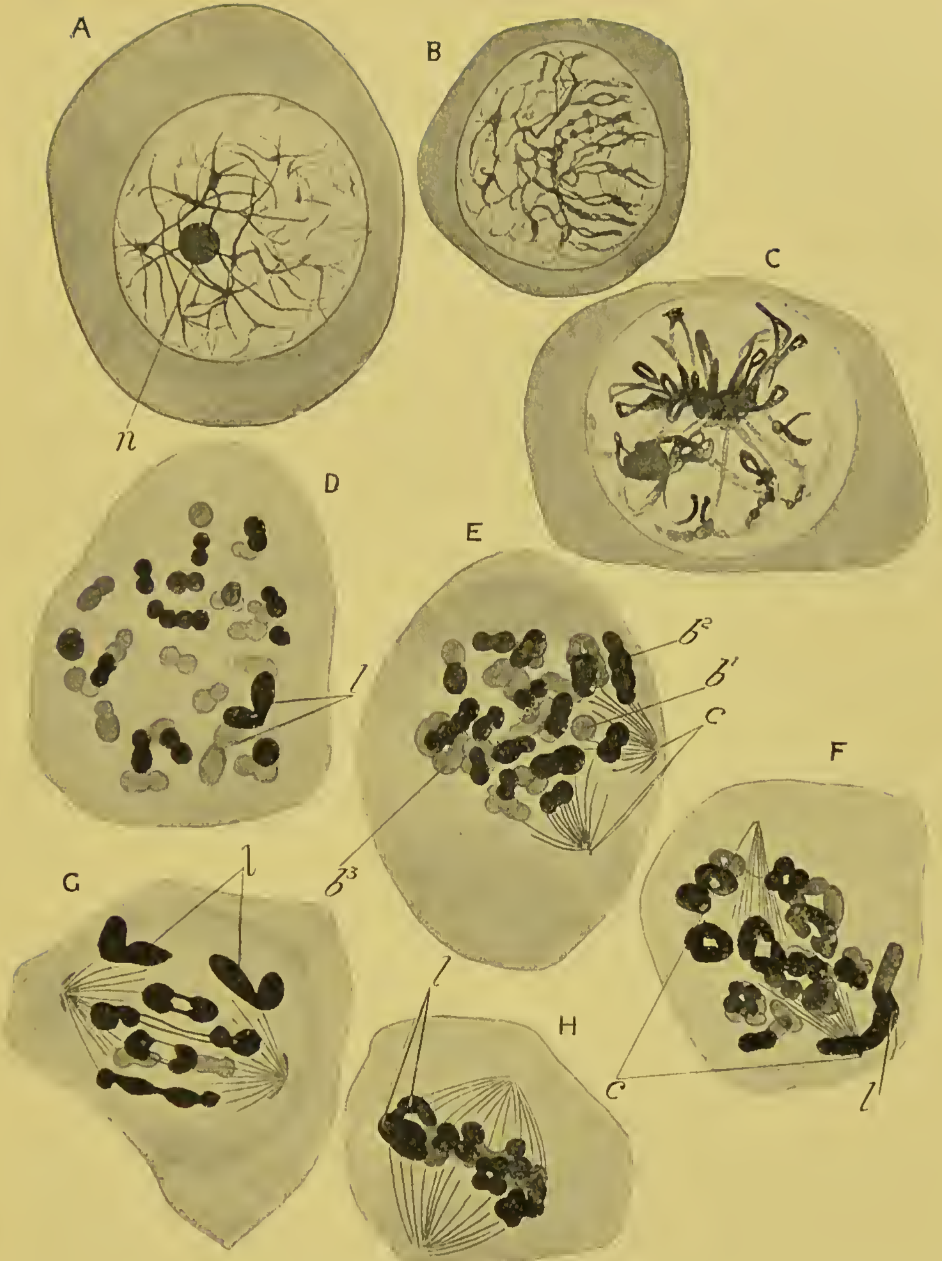

FIG. 1.—Eight views of the maturation of the male cells of *Lepidosiren paradoxa*. (After Agar.)

A, leptonema stage. B, beginning of zygonema. C, strepsinema stage, beginning of synizesis. D, separation of two chromosomes which were united in syndesis completed; nuclear membrane disappeared. E, second pairing of chromosomes begun, appearance of centrosomes for the first maturation spindle. F, later stage in second pairing of chromosomes, the centrosomes of the first spindle now situated at opposite poles of the nucleus. G, the anaphase of the first maturation division. H, the metaphase of the second maturation division. b^1, a chromosome which has not yet paired with its fellow; b^2, two chromosomes united end to end; b^3, two chromosomes united in a ring; *c*, centrosome of spindle of first maturation division; *l* (in D), the two longest chromosomes, (in F), the two longest chromosomes united end to end, (in G), the two longest chromosomes separating from each other in the first maturation division, (in H), one of the longest chromosomes dividing into two in the second maturation division; *n*, nucleolus.

This is the aggregation of the chromosomes in a kind of bunch at one side of the nucleus. This persists for a time, but eventually the chromosomes separate out again from one another, a process known as **diakinesis.** The rings of the strepsitene stage are completely broken up into their constituent parts, and the number 2 x is consequently restored by the time that diakinesis has reached its utmost limit.

During diakinesis the chromosomes become driven apart from one another and become spread out under the nuclear membrane, and the nucleolus during the same time gradually loses its staining power and disappears from view. The nuclear membrane then disappears and the nuclear sap mingles with the cytoplasm (Fig. 1, D). At the same time, the **centrosome,** which forms the centre of the polar rays in every nucleus undergoing karyokinesis, and which lies at the side of the resting nucleus, is seen to have divided into two daughter centrosomes (Fig. 1, E *c*); they are already moving apart to take up positions at opposite poles of the nucleus, whilst between them the rays of the **achromatic spindle** are already developing.

The chromosomes, which become much shorter and thicker, now join end to end in pairs; but their free ends then swing round and join one another, so that eventually parasyndesis again occurs (Fig. 1, F b^1, b^2, and b^3), and the number of chromosomes is again halved, and we thus obtain x short, thick, ring-like chromosomes which become arranged in a plane so as to form the **equatorial plate** of the first spindle.

The division of the nucleus then takes place; each daughter nucleus receives one half of each ring, *and these halves represent the separate chromosomes of the diakinetic stage which subsequently fused with another* (Fig. 1, G).

In the case of the testis the division of the nucleus is followed by the division of the germ cell into two equal cells, and in this way two **spermatocytes of the second order** are formed: the undivided germ cell with its nucleus and condensed chromosomes being termed the **spermatocyte of the first order.** The nuclei of the spermatocytes of the first order do not undergo a resting stage, but in each of them a new spindle is formed at right angles to the first, and each chromosome becomes split longitudinally so that at the ensuing division of the nucleus one half of each chromosome goes into each daughter nucleus (Fig. 1, H).

If this description has been followed it will be seen that, in the first maturation division whole chromosomes go to each daughter nucleus—this is termed the **reducing division**; whilst in the second division one half of each chromosome goes to each daughter nucleus —this is termed the **equating division.**

The division of the nucleus is again followed by the division of the cell, and in this way four cells are derived from each spermatocyte of the first order. These cells are termed **spermatids** and each spermatid becomes converted into a **spermatozoon.** The manner in which this change is effected seems to be fundamentally the same in most animals which have been examined. One of the best and most recent accounts of it is that given by Duesberg (1909), who worked on the development of the spermatozoa of the rat. According to this author the spermatid is a small polygonal cell containing a large resting nucleus, at one side of which is the centrosome which functioned in the last maturation division. This centrosome has already divided into two minute **centrioles** lying one above the other (Fig. 2, A, c^1, c). Besides the centrioles there is a peculiar body embedded in the cytoplasm termed the **idiosome,** apparently derived from the **sphere** or modified cytoplasm which surrounded the centrosome, although it is now widely separated from the centrioles. The distal or outer centriole begins to give rise to a thin filamentous flagellum which is the rudiment of the **tail** of the spermatozoon, whilst the idiosome travels to the opposite side of the nucleus from that on which the centrioles lie, and becomes applied to the nuclear membrane and forms a cap-like structure known as the **acrosome.** The nucleus sends out a prolongation which reaches the lower or proximal centriole, and this becomes applied to the nuclear membrane and forms a plate-like thickening on it. The nucleus carrying the acrosome then begins to *emerge from the cytoplasm* on the opposite side of the cell to that on which the centrioles are situated. Both centrioles are dragged after the nucleus. Round the upper or immersed half of the nucleus the cytoplasm is differentiated so as to form a funnel of clear substance called the "**ruffle**" (manchette) (Fig. 2, D, *m*). The nuclear sap is then apparently expelled and the nucleus converted into an almost uniform mass of staining matter. Its form changes—no longer spherical, it becomes sickle-shaped (Fig. 2, E). The distal centriole divides into two daughter centrioles, and to the lower and proximal of these, the tail filament, now greatly grown in length, is attached, whilst the upper or distal one forms a ring surrounding the filament. The nucleus now emerges completely from the cytoplasm. It forms the **head** of the spermatozoon; the ruffle disappears, but the acrosome is still distinguishable as a thickening on the convex side of the sickle. The cytoplasm now forms a thick mantle surrounding the lower part of the filament (Fig. 2, G). The ring-shaped centriole travels away from the nucleus along the filament till it reaches a definite position. The cytoplasm shrinks more and more and is eventually completely cast off. The ring-shaped centriole and the piece of the filament between it and the nucleus forms the **middle-piece** of the spermatozoon. When these changes are complete the spermatid is transformed into a spermatozoon, begins to exhibit active movement, and is capable of fertilizing a ripe egg.

We must now turn our attention to the process of maturation of the egg. We left the egg in the stage when the nucleus was inordinately swollen with cell sap, and when there was a large nucleolus. All the changes exhibited by the ripening spermatocyte

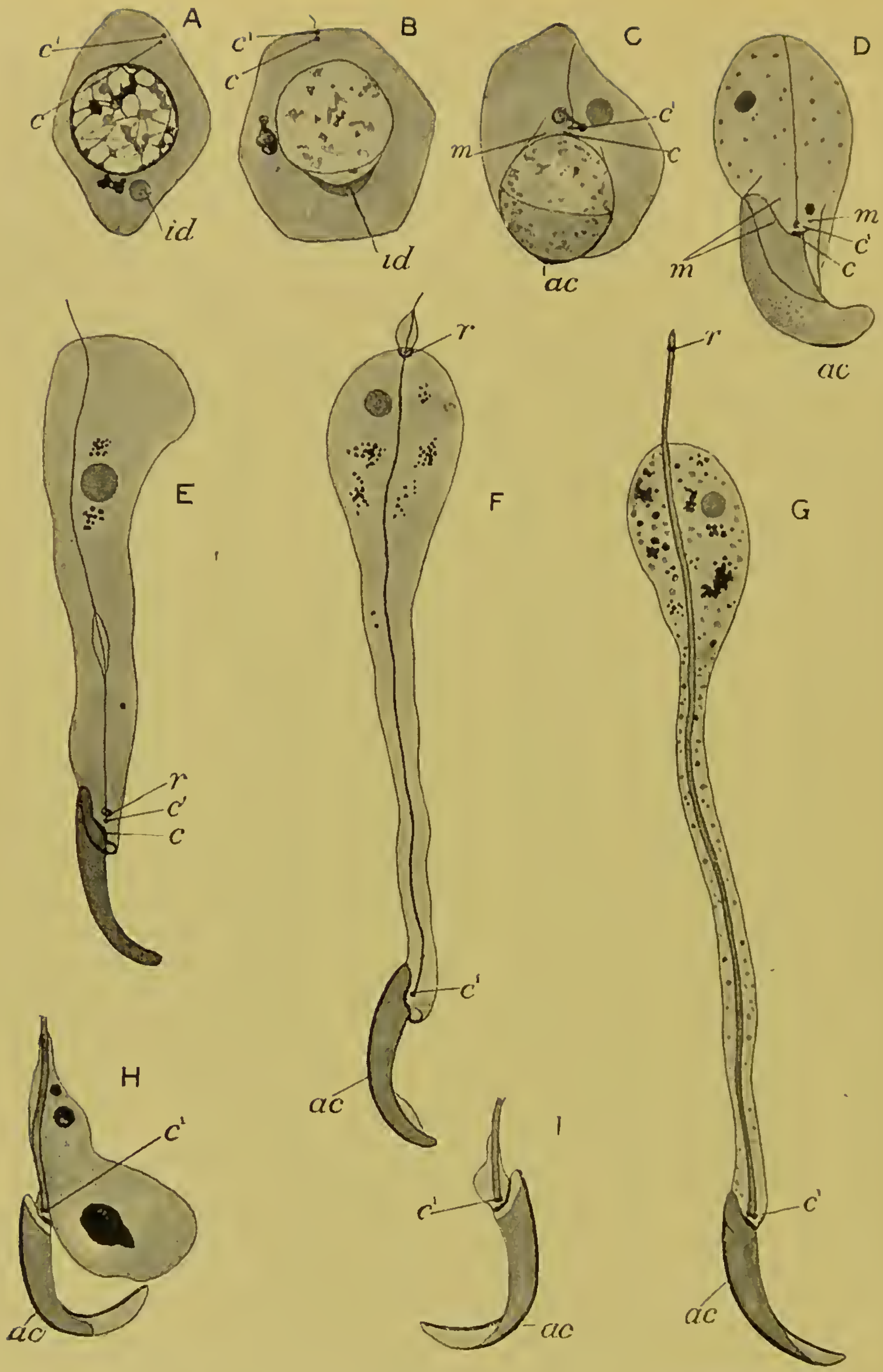

FIG. 2.—Nine stages in the transformation of a spermatid into a spermatozoon. (After Duesberg.)

A, spermatid with two centrioles, the proximal and the distal, at one side of nucleus and the idiosome at the other side. B, stage in which the distal centriole is giving rise to a filament, and in which the idiosome is applied to one side of the nucleus. C, stage in which the nucleus is beginning to emerge from the cytoplasm dragging after it the centrioles; the filament, now grown longer, is immersed in the cytoplasm, the ruffle has appeared, and the idiosome has become the acrosome. D, stage in which the nucleus has begun to become sickle-shaped. E, stage in which the distal centriole has divided into two and the outer one has become a ring. F, stage in which the ring is moving outwards along the filament. G, stage in which the ring has moved beyond the cytoplasm. H, I, two stages in casting off the cytoplasm. *ac*, Acrosome; *c*, proximal centriole; c^1, distal centriole; *id*, idiosome; *m*, ruffle (manchette); *r*, ring centriole.

are exhibited also by the **oocyte of the first order,** as we term the egg when it has reached its full size. Its nucleus passes through the stages of leptonema, zygonema, pachynema, strepsinema, etc. The nucleolus disappears, the nuclear membrane dissolves, and the nuclear sap mingles with the cytoplasm. The consequence of this is, that since the chromosomes form a small and inconspicuous mass, the egg, viewed under the low power of the microscope, appears to have lost its nucleus, and this is a ready way to distinguish unripe from ripe eggs, or in other words, oogonia from oocytes of the first order.

The great difference between oocytes and spermatocytes of the first order is that in the case of the latter when the cell divides, it gives rise to two daughter cells of the same size which form spermatocytes of the second order, but when the oocyte divides, it forms two daughters of unequal size, the larger forming the **oocyte of the second order** whilst the smaller forms a rudimentary **cell** incapable of development, termed the **first polar body.** At the second maturation division the same phenomenon repeats itself, the oocyte of the second order divides into two unequal daughters—the larger is the **ripe ovum,** whilst the second is again a rudimentary cell which never develops and which is termed the **second polar body.** The first polar body often divides into two daughters of equal size which are, like the second polar body, to be regarded as sisters of the egg, or better, as rudimentary eggs (Figs. 3, B, and 4, B). The same sacrifice of quantity to quality therefore which is seen in the absorption of many oogonia by their more fortunate sisters, repeats itself in the maturation divisions.

The ripe egg can now receive the spermatozoon. As soon as the head of the first spermatozoon has penetrated the egg an alteration of the surface of the latter usually takes place, which cuts off the tail of the spermatozoon and prevents any other spermatozoa from entering; the middle piece of the successful spermatozoon, however, follows the head into the egg. This alteration in the surface of the egg has been diagnosed by Loeb as a kind of cytolysis: for it can be observed that a number of fine globules issue from the surface of the egg, and that their outer surfaces coalesce to form the inner egg-shell or **vitelline membrane.**

Meanwhile the spermatozoon head within the ovum swells up, it assumes a vesicular form, and nuclear membrane, cell sap and chromosomes can be again demonstrated in it. The middle piece takes on the character of a centrosome and around it the achromatic rays appear, forming what is termed an "**aster.**" The **male pronucleus,** as the spermatozoon head is now termed, moves towards the residual nucleus of the egg, termed the **female pronucleus,** which in its turn advances towards the male. Male and female pronuclei then meet and fuse and form a single nucleus, the **zygote nucleus,** and fertilization is complete.

After a resting period of an hour or two the zygote nucleus begins to prepare for karyokinesis, and the spindle is so formed that its equatorial plate lies at right angles to both female and male pronuclei, and so both are halved at the ensuing division and equal parts of both distributed to the first two cells into which the ovum

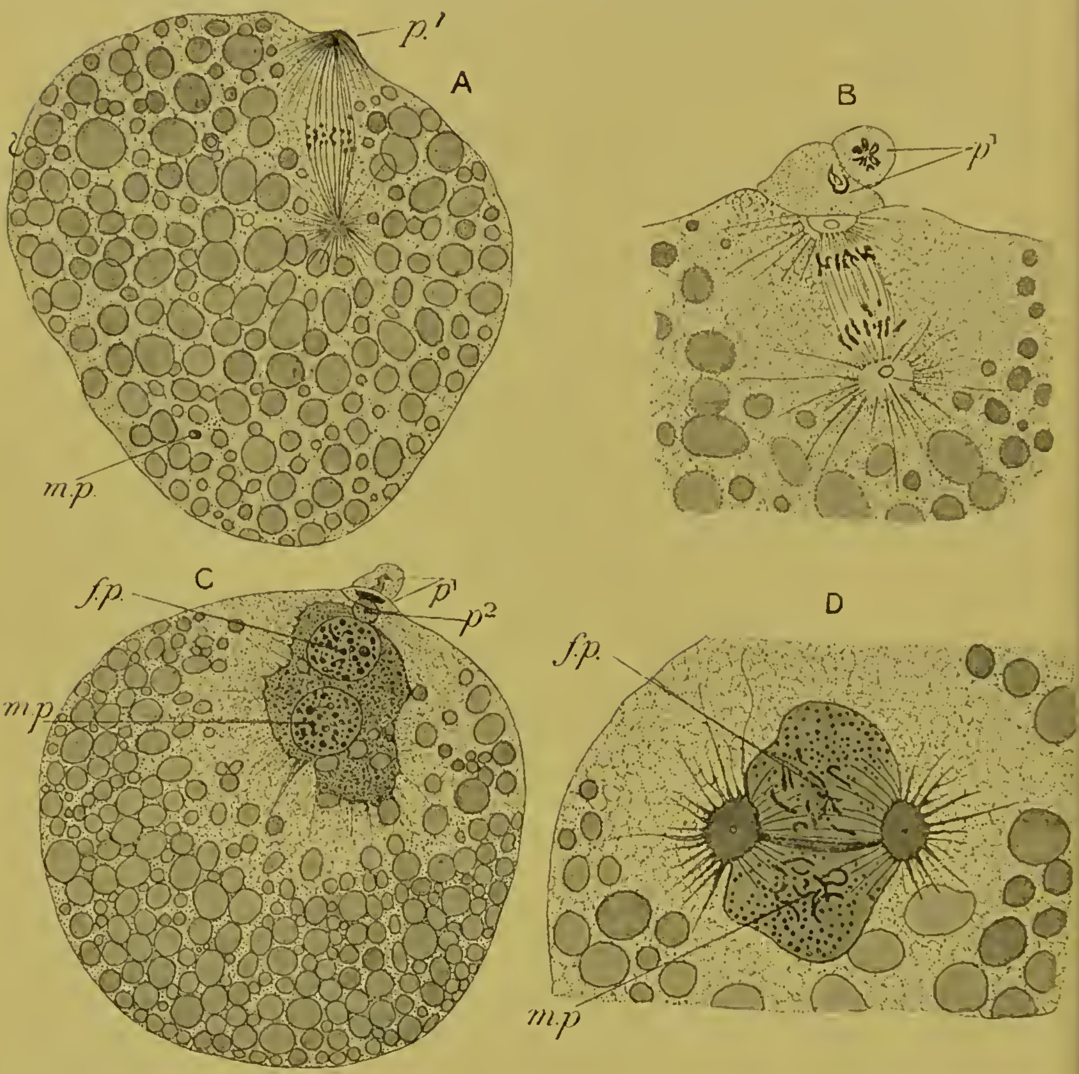

Fig. 3.—Four stages in the maturation and fertilization of the egg of *Crepidula plana*. (After Conklin.)

A, formation of first polar body; the spermatozoon has entered the egg and has begun to swell up into the male pronucleus. B, formation of second polar body; the first polar body has divided into two. C, the male and female pronuclei have come together. D, formation of the first cleavage spindle; the female pronucleus above and the male pronucleus below are still clearly distinguishable from one another. *fp*, Female pronucleus; *mp*, male pronucleus; p^1 (in A), first polar body, (in B and C), two cells resulting from division of first polar body; p^2, second polar body.

divides. In some few cases it is possible to distinguish in this first equatorial plate the chromosomes derived from male and female pronuclei respectively, for they are of different sizes and arranged in two different groups. This is especially clear in the egg of *Crepi-*

dula plana, a species in which the processes of maturation and fertilization have been worked out in great detail by Conklin (1902). The centrosomes of the first cleavage spindle are here stated to be derived from the division of the sperm centrosome. It is therefore reasonably certain that in all cases each daughter cell receives a half of each male and female chromosome.

It is by no means always true that the spermatozoon can only enter the egg after the formation of both polar bodies. In many cases it enters the egg whilst it is still an oocyte of the first order, and even before the nuclear membrane has been dissolved and the germinal spot has disappeared. This is true of the eggs of many Annelida and Mollusca. In other cases, such as in some Ascidians, the first maturation spindle is formed before the spermatozoon enters, but the first maturation division is not completed till the spermatozoon is inside the egg. Finally, in *Dinophilus* according to Shearer (1912), the spermatozoon enters the oogonium and remains passive during the growth and maturation of the germ cell.

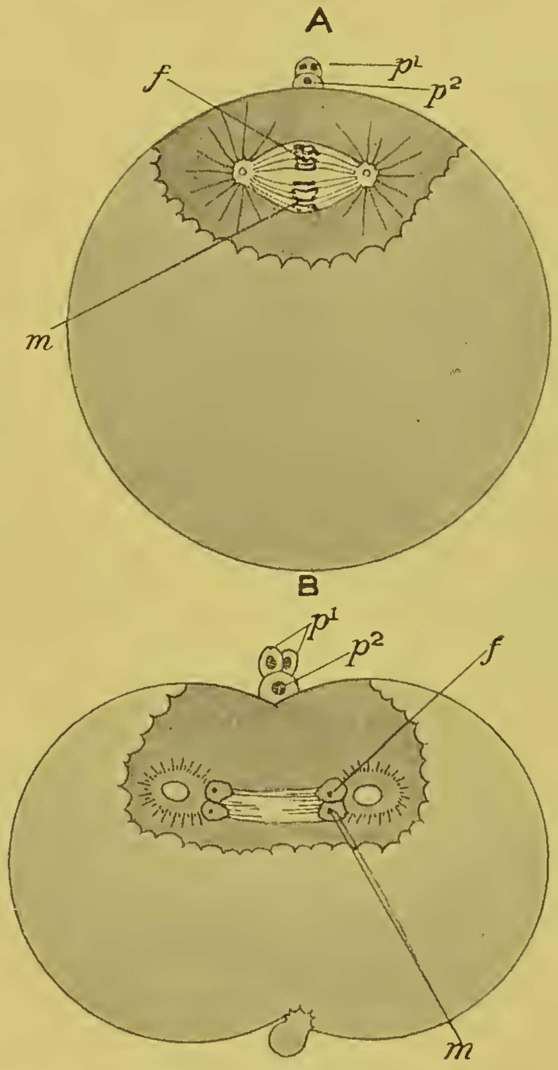

Fig. 4.—Two stages in the first division of the fertilized egg of *Crepidula plana*. (After Conklin.)

A, the first cleavage spindle; female chromosomes above separated by an interval from male chromosome below. B, the division of the zygote nucleus is complete. *f*, Female chromosomes; *m*, male chromosomes; p^1 (in A), first polar body, (in B), products of division of first polar body; p^2, second polar body.

If the eggs are stale, *i.e.* if they have been shed too long from the ovary before being fertilized, then more than one spermatozoon can enter them, and an extra centrosome is thus introduced, between which and one or both of the centrosomes resulting from the division of the centrosome of that sperm which has actually effected fertilization, extra achromatic fibres can be developed and irregular division of the egg results; in most cases this takes the form of simultaneous division into four equal parts.

In the case of large eggs, like those of birds, it appears that normally a considerable number of spermatozoa enter the egg. One only unites with the female pronucleus and forms the zygote nucleus, the rest divide independently and form groups of small cells which are produced by the aggregation of the cytoplasm round the products of their division. Soon, however, the cells formed round the daughters of the zygote nucleus crush out and kill these other cells, and the former alone enter into the formation of the embryo.

It appears therefore that the alteration of the surface of the egg so as to exclude supernumerary spermatozoa, which is so marked a feature in small eggs, must be due to some chemical influence radiating from the zygote nucleus, and that in large yolky eggs it does not reach sufficiently far to prevent the entry of extra spermatozoa at some distance from the first one.

The sequence of events worked out by Agar for the maturation of the nuclei in the male cells of *Lepidosiren* agrees fairly closely with that described by other workers for other forms. But in many cases, before the first maturation division has taken place, when in the paired or **bivalent** chromosomes the components are beginning again to separate, a longitudinal split appears at right angles to the split separating the components; this is the anticipation of the final division of each chromosome into longitudinal halves which occurs in the second maturation division. We thus get quadripartite chromosomes, which are termed **tetrads**. Further, many workers maintain that in the final pairing of chromosomes which takes place just before the first maturation division, there may in some species be an end-to-end junction, **metasyndesis**, instead of a side-to-side junction or parasyndesis.

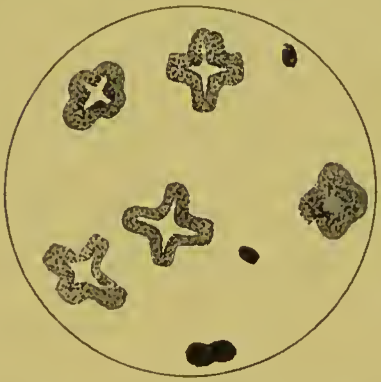

Fig. 5.—Polar view of the first maturation division of the male germ cells of *Alydus pilosulus*, in order to show **tetrads**. (After Wilson.)

When a substance like chromatin appears in the ripening eggs and spermatozoa, in exactly the same forms, generation after generation, and when the masses of chromatin continually undergo complicated changes of shape in the same order, it is natural to imagine that such a substance must be of great importance in the main function of the germ cells, *i.e.* transmitting the hereditary qualities of the parents; as a matter of fact it was on the casting off of the two polar bodies as a preliminary to development, and on the nuclear changes which accompany this phenomena, that Weismann (1886) founded his famous theory of heredity. According to him the nucleus of the egg was supposed to have a portion of its material charged with the function of producing the peculiarities of the cytoplasm of the unripe egg—the amount, colour, and composition of the food-yolk, etc. When this task was accomplished and the ovum was ripe, it was supposed that this portion of the nucleus, termed by Weismann **histogenetic plasm,** was extruded as the first polar body. The extrusion of the second polar body he explained by his theory of "**ids**."

According to this theory, the material basis of the transmission of the parental qualities to the child is contained in the chromatin which is organized into a number of "ids." Each "id" contains within itself the whole potentiality of the animal, *i.e.* one "id" alone is capable of causing the egg to develop into an adult animal. The "ids" are similar, but not exactly the same, and the animal which develops is a compromise between the potencies of the various "ids." The "ids" are capable of assimilation and growth, and in each longitudinal division of the chromosome each "id" contained therein is divided into two precisely similar daughter "ids," but in the transverse or reducing division (which in Weismann's day was supposed to be the second, not the first division) different "ids" are separated from one another.

This is what happens in the formation of the second polar body, and now the spermatozoon brings in an equal number of "ids" and thus the number originally present in the oogonia is restored. Since the "ids" of the spermatozoon are not exactly the same as those of the egg, and since Weismann assumed that the casting forth of half the maternal "ids" might take place at random, *i.e.* might consist of any group of the "ids" amounting to half the number, the basis was given for inheritable variations, because different combinations of maternal and paternal "ids" might come about by a difference in the "ids" which were cast forth at the reducing division.

It is one great merit of Weismann's theories that, whatever may be thought of their truth or untruth, they have acted as powerful stimulants to research. Hertwig (1890) published a work on the spermatogenesis and oogenesis of the worm, *Ascaris megalocephala*, in which he showed, for the first time, the parallelism in the changes which occur during the ripening of both kinds of germ cell, and proved that the polar bodies were the degenerate sisters of the egg, thereby overthrowing Weismann's theory of the histogenetic plasm. But Weismann's theory of the meaning of the reducing division was not thus disproved, although of course it was shown that it was the first, not the second maturation division in which whole chromosomes were separated from one another, and to which Weismann's hypotheses must apply. This theory was shattered by the work which ensued on the discovery of what have been called **sex-chromosomes,** by McClung, Wilson, and other American workers. Wilson has given an excellent summary of the whole subject (1911), and to this we must now address ourselves.

In the spermatids of certain insects it was observed that in some cases there was one more chromosome than in others. Let us denote these numbers by the formulae x and $x+1$. Further investigation revealed the fact that this extra chromosome, termed the **accessory chromosome** or **hetero-chromosome**, does not pair with any other chromosome before the first maturation division: in this division it divides longitudinally into two, whilst in the *second* maturation division it does not divide at all, but passes to one pole of the spindle, and is thus distributed to *one* of the two spermatids resulting from that division (Fig. 6). All the ripe eggs showed a number of chromosomes equal to the number in those spermatids which had the extra chromosome.

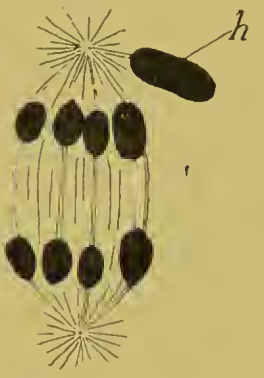

Fig. 6.—Second maturation division of the male germ cells of *Protenor belfragei*. (After Wilson.)

h, Accessory chromosome.

The zygotes which resulted from the conjugation of these eggs with the two kinds of spermatozoa would show therefore $2x+2$ and $2x+1$ chromosomes in their nuclei. Examination of the tissues in the adult insect showed that the dividing nuclei of males possessed $2x+1$ chromosomes, whilst those of females had $2x+2$ chromosomes. It seemed, therefore, probable that in the case of these insects, this abnormal chromosome contained some material which determined the production of the female sex in the zygote to which it passed.

In other cases it was found that the spermatids all contained an abnormal chromosome, but that this abnormal chromosome was in some spermatids large and in others small. It was shown that in the spermatocyte of the first order both abnormal chromosomes were present in the same nucleus, and that they both divide in the first maturation division by longitudinal splitting; but that before the second division they fuse together to form a bivalent chromosome, and that in the second maturation division they again separate from one another, and that one proceeds to each daughter nucleus; so that in the case of these chromosomes alone the second maturation division is a "reducing division," whereas in the case of all the others the first maturation division is the reducing division. These abnormal chromosomes are termed **idiochromosomes** (Fig. 7).

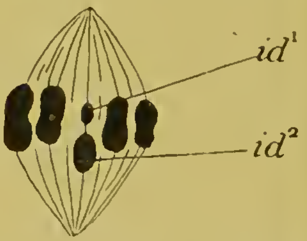

Fig. 7.—Second maturation division of the male germ cells of *Euschistus variolaris*. (After Wilson.)

id^1, the small idiochromosome; id^2, the large idiochromosome.

When two idiochromosomes are present, the developing eggs always carry the larger one when maturation is complete, and the nuclei in the tissues of the adult female have two large special chromosomes; whereas the nuclei in the tissues of the adult male carry one large and one small idiochromosome. Hence it is evident that the female grows from a zygote which has resulted from the fusion of an egg with a spermatozoon which contained the larger idiochromosome; the formula of its nuclei therefore will be $2x + 2a$, where a denotes the large idiochromosome. The male on the other hand has developed from a zygote which has resulted from the fusion of an egg with a spermatozoon containing the small idiochromosome, and the formula of its nuclei will be $2x + a + b$, where b denotes the small idiochromosome. Hence the presence of two a chromosomes in the zygote determines the formation of a female, and we can reduce the case of idiochromosomes to that of the hetero-chromosome by supposing b diminishes until it disappears altogether.

Other modifications have been described by Wilson; thus a may be represented by a group of chromosomes, but this group acts as a unit in the reducing division and passes to one of two daughter spermatocytes. The principle therefore is the same, and Wilson, in his final summary, suggests that what determines a zygote to be a female is an *excess* of peculiar trophic chromatin in its nuclei. Whilst a zygote which is characterized by a defect in this regard becomes a male.

No such clear cases of differences between the sexes in the number of chromosomes have been found outside insects. Many statements as to the existence of such a dimorphism in other groups have been made but have not been proved to be true. It is quite obvious that such a dimorphism can only be demonstrated where the number of chromosomes is few and easily counted, and that where they are numerous the matter must remain in doubt.

It is clear that the existence of special sex-determining chromosomes is irreconcilable with Weismann's conceptions of the chromosomes as bundles of equipotential "ids" closely resembling one another, any one of which was able to direct the whole development.

But the discovery of what Wilson calls **sex-chromosomes** has led to other results of a far-reaching character. The reducing division is a separation of whole chromosomes which immediately before this had paired with one another. Now the idiochromosomes, when present, always pair with one another, and the question arises whether the pairing of the other chromosomes is not just as definite a matter as that of the idiochromosomes. An examination of cases like the germ cells of insects, where the number of chromosomes is small, reveals the fact that in the nucleus, before "pairing" has taken place, the chromosomes are of different sizes, but that there are always—except in the case of idiochromosomes—two chromosomes of the same size, and that these two pair with one another. The pairing therefore, and the subsequent distribution of the members of the pair to different gametes at the reducing division, is a definite and not a haphazard phenomenon, and Montgomery (1904), to whom we owe this important observation, has suggested that the two homologous groups of chromosomes, which he asserts can be seen in all the nuclei of the body, are derived from the male and female gametes respectively, to the union of which the adult, which produces the germ cells, owes its origin.

We thus reach the conception that male and female chromosomes remain side by side without fusing in the nuclei of the offspring during all its life, but that when this organism in turn produces germ cells these two kinds of chromosomes are segregated into different gametes. Now this conclusion appears at first sight to accord exactly with the theory to which the followers of Mendel have been led, and it entirely destroys the second half of Weismann's theory of the production of variations at the reducing division, by the casting out of half the chromosomes, selected at random.

This brilliant school of "Mendelians," whose labours have been summarized by their most brilliant member, Bateson (1909), have been led to conclude that when two strains of animals are crossed, the hybrid produces two kinds of spermatozoa, or ova, as the case may be, one carrying the characters of the male and the other of the female with respect to each differentiating character.

We should be wanting in our duty, however, if we allowed our readers to imagine that Montgomery's theory had been fully established. It is, on the contrary, only in the stage of a working hypothesis, and it labours under many difficulties. Thus, its superficial agreement with the Mendelian theory disappears under a deeper analysis. On the idea that male and female chromosomes are distributed to distinct gametes, each zygote should produce only two kinds of gametes (leaving out of sight the sex-chromosomes for the present), one with the maternal, one with the paternal characteristics. But the Mendelian hypothesis demands two kinds of gametes with regard to *each* differentiating character. Thus if a pea-plant have round and green seeds, and if it be crossed with another having yellow and angular seeds, we must expect the hybrid plants to produce seeds which give rise to plants bearing yellow and green seeds, and round and angular seeds; but all the yellow seeds will not be angular nor will all the green be round; on the contrary there will be *four* categories of seeds produced, viz.:—yellow round, yellow angular, green round and green angular. It is not easy to see how Montgomery's hypothesis can be fitted in with this breaking up of the parental hereditary potencies into factors which are distributed among the germ cells independently of one another. Agar has, however, pointed out that in the stage of zygonema, when the first pairing takes place between chromosomes, there is opportunity for the exchange of material between them, and that when they again separate in the stage of strepsinema they may be different from what they were before the pairing took place.[1]

Montgomery's theory demands, further, the belief that the identity of each chromosome remains unimpaired during the resting period of the nucleus, when no trace of distinct chromosomes can be detected.

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[1] It has also been suggested that, previous to the reducing division, the bivalent chromosomes, each of which (*ex hypothesi*) consists of a paternal and of a maternal chromosome joined end to end, may not all be arranged so that their homologous ends point in the same direction. If this were so, one gamete might receive one chromosome from one parent and one from another.

Occasionally, it is true, the sex-chromosome remains distinct during the resting period. We may imagine, by an act of faith, that the others too retain their identity although we cannot see them, but it seems to us that the only meaning which can be given to such an identity would be the persistence of a centre for the synthesis of some special substance. Lastly, it may be that in some cases sex is not irrevocably determined in the germ, but can be determined by feeding. This at any rate has been asserted by Born (1881) in the case of tadpoles—though his results have not passed unquestioned. Wilson attempts to get over this difficulty by supposing that the sex-chromosome is only one of the factors which determine sex.

Our final conclusion is that investigators have only touched the fringe of an intensely interesting and important subject, and that a great deal more research must be done before definite conclusions can be arrived at.

The meaning of the process of fertilization has proved a fascinating subject for speculation. That the union of the two nuclei is not *per se* necessary for development, is proved by the experiments of Loeb and his pupils, who have caused the unfertilized eggs of Echinodermata, Annelida, and Mollusca to go through the early stages of their development by increasing the salinity or alkalinity of the water in which they lie, *i.e.* by immersing the egg in what is called a hypertonic solution, or by causing the egg to form a vitelline membrane by rapid treatment with butyric and similar organic acids. By uniting these two methods, *i.e.* by first causing the egg to form a membrane through treating it with butyric acid and then treating it with a hypertonic solution, a close approach to normal development may be attained.

The formation of the **vitelline membrane** is due, according to Loeb, to incipient cytolysis, *i.e.* the peripheral protoplasm breaks down, forming minute globules which cohere together so as to form the membrane. Too long exposure to the acid causes the egg to die, by a continuation of the same process until the whole cytoplasm is resolved into a mass of globules, but this process is arrested by the action of the hypertonic solution. Therefore Loeb concludes that the influence of the spermatozoon is primarily chemical; in fact he supposes that it carries into the egg a ferment, "lysin," which starts the process of cytolysis, and also another substance which arrests this process after it has resulted in the formation of a membrane.

Loeb himself and his pupils Godlewski (1906) and Kupelwieser (1906) have shown that it is possible to fertilize the eggs of a Sea-urchin with the sperm of a Crinoid, a Star-fish, and even of a Mollusc. In these cases the resulting organism, as long as it lives, resembles exactly the normal larva which would have resulted if the egg of the Sea-urchin had been fertilized by its own sperm, and betrays not the smallest trace of the hereditary influence of the foreign sperm which was used to evoke development. But of course, throughout the animal kingdom, offspring are as likely to resemble the male parent as the female, and there must come a point when the hereditary influence of the male asserts itself. This, Loeb suggests, is when the foreign chromatin becomes dissolved and spreads its influence through the cytoplasm.

Now the common experience of breeders, as collected by Darwin, bears witness to the beneficial effect on the vigour of the offspring which is gained by crossing two parents of slightly different strains. Their experience, moreover, is, that in what is termed **inbreeding**, that is, when the male is nearly related to the female in blood, the resulting offspring exhibit weakness of constitution. In the case of the higher plants, which have both kinds of germ in the same individual, self-fertilization produces in many cases similar results. This can be explained if we imagine that in the normal multiplication of the cells in a developing organism, starting from the zygote and leading through many cell-divisions to the formation of germ cells, the wastage of the original chromatin is not quite made good.

From experiments made on Protozoa we conclude that without chromatin no assimilation or building up of fresh living material can take place, and we can only imagine that the influence of chromatin is exerted through the substances which it is constantly giving off into the cytoplasm. Now if our assumption be just, the germ cells of each generation should become more and more imperfect in their chromatin equipment, and this imperfection should exhibit itself as a diminution in vitality. Hence we conclude that the prime object of conjugation is to maintain the vitality of the stock by adding together two chromatins of slightly different kinds, which will presumably not be deficient in the same places. On this view the purpose of reproduction by unicellular germs would be to render it possible for the two chromatins to be thoroughly mixed.

Herbst (1900) has started an egg to develop by using hypertonic solutions and valerianic acid, and then, when the nucleus had divided into two, he has fertilized the bicellular organism with spermatozoa. As a result, one of the two nuclei has conjugated with the sperm nucleus, and an organism was produced, on one side of purely maternal character and on the other side showing the paternal influence.

It has been asserted in contradiction to this that there are some plants, such as the Pea, which normally fertilize themselves and yet undergo no deterioration, and others like *Hieracium*, the hawkweed, in which sexual reproduction has been entirely replaced by asexual. But such isolated cases cannot be allowed to weigh in the balance against the great mass of evidence which tells in the contrary direction. The deterioration due to inbreeding may be very slow in showing itself, and a cross at long intervals may be sufficient to restore vitality; and he would be a rash man who would deny this possibility in the case of any of the species which apparently undergo continuous self-fertilization.

Leaving now the question of the meaning of the sexual element in development, and turning to the developmental process itself, we find that every animal passes through two phases in its passage to the adult form. In the first of these phases the young organism is sheltered from external influences either by an egg-shell or by the body of the parent, or by both. Its food is supplied in the first instance by the **deuteroplasm** or **food-yolk** embedded in its own substance, supplemented in many cases by maternal secretions. In the second phase the young animal, after escaping from its shelter, is obliged to seek its own food, but it never is exactly like the parent, and some time elapses and considerable growth takes place before it attains the adult form. In the first phase it is known as an **embryo**, in the second as a **larva**.

In different life-histories the embryonic and larval phases vary enormously in their relative lengths. Sometimes, as in Echinodermata, the young organism is thrown on its own resources at an extraordinary early period of development, a very long larval life ensues, and the young animal is at first utterly unlike the parent. In other cases, as in the case of Man, when the young organism leaves the parent it resembles the adult in all essential features. In such cases it is customary to say that the larval stage has been omitted. But the practice of confining the term "larva" to cases where the free-living young differ markedly from the parent is not logical. The baby is very different from a full grown man, and so is the young child; for example, the proportions of the limbs are markedly different, and a continuous series of stages can be found between differences of this kind and differences as great as those which divide the larva from the adult Echinoderm. We may assert with confidence that all animals pass through first an embryonic and then a larval phase of development, and nothing is gained by calling a larval stage which closely resembles the adult a "brephic" or "neanic" stage, as was originally suggested by Hyatt and has been adopted by some English zoologists.

Of course, development goes on throughout both embryonic and larval phases, and the form of the organism is constantly changing; but there is one great group of animals, the Arthropoda, in which the organism is confined within a rigid envelope derived from its own secretions, and in this case, for a definite period of time, the external form appears to be *unchanged*; only when the dead envelope is burst and cast off, do the internal changes which have been going on manifest themselves in a change of form. Hence we can appropriately speak of these periods of fixity of form as a **series of larval stages**, or, as Sharpe (1895) has suggested, we might call them **instars**. In other groups of the animal kingdom where this rigidity of form does not obtain, there are, nevertheless, crises in development when great changes take place very rapidly, accompanied in many cases by the casting off of portions of the body of the larva. These crises are termed **metamorphoses**, and the stages of quiet growth preceding and succeeding them are looked on as stationary in comparison, and spoken of as larval stages or instars.

Now a great deal of the interest in the science of embryology has arisen from the fact that both the embryonic and larval phases of development show features which have been interpreted as being a reproduction of the characters of far-off ancestors of the species to which the adult belongs. This theory is the so-called **fundamental law of biogenetics,** and is summed up in the phrase, "*The individual in its development recapitulates the development of the race.*" If this "law" can be substantiated the interest in embryology becomes immense, it binds all the innumerable phenomena of development into one coherent scheme, and opens the door to the hope that we may yet be able to sketch the main history of life on the earth.

The direct evidence of this history, as contained in the fossil record, takes us back only a short distance. In the lower Cambrian rocks the great groups of Mollusca, Arthropoda, Echinodermata, and Brachiopoda are all as sharply marked off from one another as at the present day, and since only hard parts are preserved, the all-important "soft" parts which constitute the real living matter are irrecoverably lost, and no trace is left of an organism except it possessed some kind of skeleton. But an egg has many points of resemblance to the simplest animals, the Protozoa, and if development be really a recapitulation of ancestral history, then the whole of the ancestral history of an animal, from the Protozoan stage to the present, should be presented in outline in its life-history.

But although most naturalists would agree that a life-history contains ancestral "elements," all would be emphatic on the subject that it likewise exhibits many features which are purely secondary, and in no way reflect the characters of ancestors. With these general considerations the agreement stops. As the founder of the Naples Biological Station has caustically remarked, it is a curious fact that every investigator is convinced that the type which he is studying has a monopoly of most of the primitive features, and that other types are secondarily modified. The endless wrangles about primitive and secondary features, which have made up so much of embryological writing for the past forty years, have so disgusted many leading workers in this field, that they have been inclined to go in the opposite extreme, and deny altogether the "biogenetic law." Driesch may be mentioned as an example of this, and a very searching criticism of the whole hypothesis is given in the Darwin memorial volume by Sedgwick (1909), who in former years had done more than most workers to illuminate the hypothesis.

Driesch's (1907 and 1908) criticism leads him to the position that the development of an egg into an adult is not to be explained by physical and chemical laws, and he therefore attributes to each species of animal a peculiar "entelechy" or soul, which presides over the task of making its germs develop. Thus we are brought back to pre-Darwinian days, to a position indeed more primitive than that of the early nineteenth century, for it is surely easier to conceive of an All-embracing Intelligence, Whose myriad plans were realized in the different species, rather than of millions of uncaused and unrelated intelligences. Why, if the entelechy of a *Strongylocentrotus* be entirely distinct from that of an *Echinus*, should their products so resemble one another? Has family resemblance in the animal kingdom no meaning? Our fathers attributed it to the Will of the Creator. Darwin taught us to believe that it was due to descent from a common stock. Driesch offers no explanation whatever, and it seems to us that this final result is the *reductio ad absurdum* of his whole system. Driesch's whole history has been that of the rebel against accepted opinions, and in so far his intervention is healthy, for nothing must be regarded as finally fixed, but pure reaction is equally unjustified.

Sedgwick's position (1909) is different. He formerly accepted the biogenetic law, but as its application seemed to yield the most discordant results, he has been led to undertake a critical examination of the assumptions on which it is based. He points out that it is tacitly assumed that when a new feature appeared in the history of the race this showed itself only in the adult condition, whilst the previous adult condition was retained as a developmental stage; that, in a word, as evolution has proceeded the life-cycle of living matter has become more complex. Against such an assumption he points out that a careful examination of the embryos of related species force us to the conclusion that new features can appear at all stages of the life-history, and that as all living matter known to us undergoes cyclical changes, it is quite open to us to assume that this has always been the case since the first appearance of living matter on the globe, and that therefore the life-cycle has been modified but not extended. Neither of these conclusions can be gainsaid *a priori*, and it is therefore time to take stock of our data.

It would lead us altogether too far to discuss the general proposition that zoological affinity means blood-relationship: this, we take it, has been abundantly proved by the evidence, which Darwin has collected, of the relationship which breeds, varieties, and species sustain to one another; if this be so it will be conceded that this zoological affinity can be exhibited just as well by embryos as by adults, and that, therefore, for the elucidation of biological affinity the study of comparative embryology is necessary even if the biogenetic law be baseless.

On looking into the question of the validity of this law, the first question which presents itself for solution is the mutual relationship of the embryonic and larval phases. On this subject Sedgwick himself (1894) threw light some years ago, when he pointed out that the embryonic phase is the remnant of a former larval phase, and that the ancestral features which it exhibits are therefore features of a former larva; but these larval features, whether ancestral or not, consisted of organs adapted to the *larval mode of life*. If, then, these features were really ancestral, what was being reproduced were primarily adaptations to an *ancestral environment.*

The proof that the embryonic stage is a concealed larval one exists widespread in the animal kingdom. When we find that the Nauplius stage of the shrimp *Penaeus* lives as a minute self-sustaining organism, using the tiny hooks at the bases of its second and third appendages as jaws to seize its prey; and that the corresponding stage in the development of the crayfish is passed within the egg-shell, but that the embryo has the Nauplius limbs in the condition of useless stumps, although, just as in the case of the free-swimming larva, the passage into the next stage of embryonic life is initiated by a shedding of the cuticle, then we have no doubt which is the more primitive, the larva or the embryo. So too, when we find in the development of the Martinique toad *Hylodes* that the embryo within the egg has the tail of a tadpole which is never used for swimming but is absorbed directly the animal hatches, we have no difficulty in concluding that the original condition of affairs was that in which the tadpole used its tail for the purpose for which its shape is adapted.

Now the phase preceding the attainment of the adult form is always larval (it is often termed **brephic** or **neanic**), and this, according to the biogenetic law, should represent the last stage which the race has passed through before attaining its present condition, and will therefore be, generally speaking, the least modified stage in the life-history, since it is the most recently added to the series. Is there, then, evidence that this stage is of ancestral significance?

The answer to this question is that there is abundant evidence of it, and a few instances of this may now be given.

The Oyster (*Ostrea*), contrary to the custom of the majority of bivalves, lies on one side, and remains fixed thus through life; but the American species, at least in its so-called "brephic" stage, when it has terminated its free-swimming existence, creeps about for a short time, and possesses, like other bivalves, a "foot," which is totally wanting to the adult oyster.

Speaking broadly, when we examine the life-history of any aberrant member of a well-defined group in the animal kingdom, we find that in a late stage of its life-history it resembles the normal member of the group to which it belongs. *Portunion,* an Isopod parasitic on Crustacea, is distorted out of all resemblance to an Isopod, but when young it is an unmistakable Isopod, a trifle simplified in structure. The Crinoid *Antedon* when adult is devoid of a stalk, and swims by muscular movements of its arms; but when it is young it possesses a stalk like the vast majority of its congeners. The Plaice, *Pleuronectes,* swims with one side down, and both eyes are twisted on to the upper side; yet the larva of this fish has both sides symmetrically formed with the eyes in the normal position, like the vast majority of Teleostei. This list could be extended indefinitely, and if the animals named are rightly classified in the groups in which they are placed, it is thereby implied that they once possessed the normal features exhibited by the typical members of these groups; and therefore, beyond all question, their late *larval stages must be of an ancestral character.*

It follows, then, that advances in evolution do, as a rule, manifest themselves when the animal is fully adult. But recent research in the laws of heredity has rendered it almost certain that inheritable variations are only those which affect the nature of the germ cell, and most zoologists refuse to believe that the adoption of a new mode of life by an adult could directly affect its germ cells. Lamarck's idea that the change in the body induced by new habits could effect a corresponding change in the germ cells is rejected by them. How, then, is the adaptation effected?

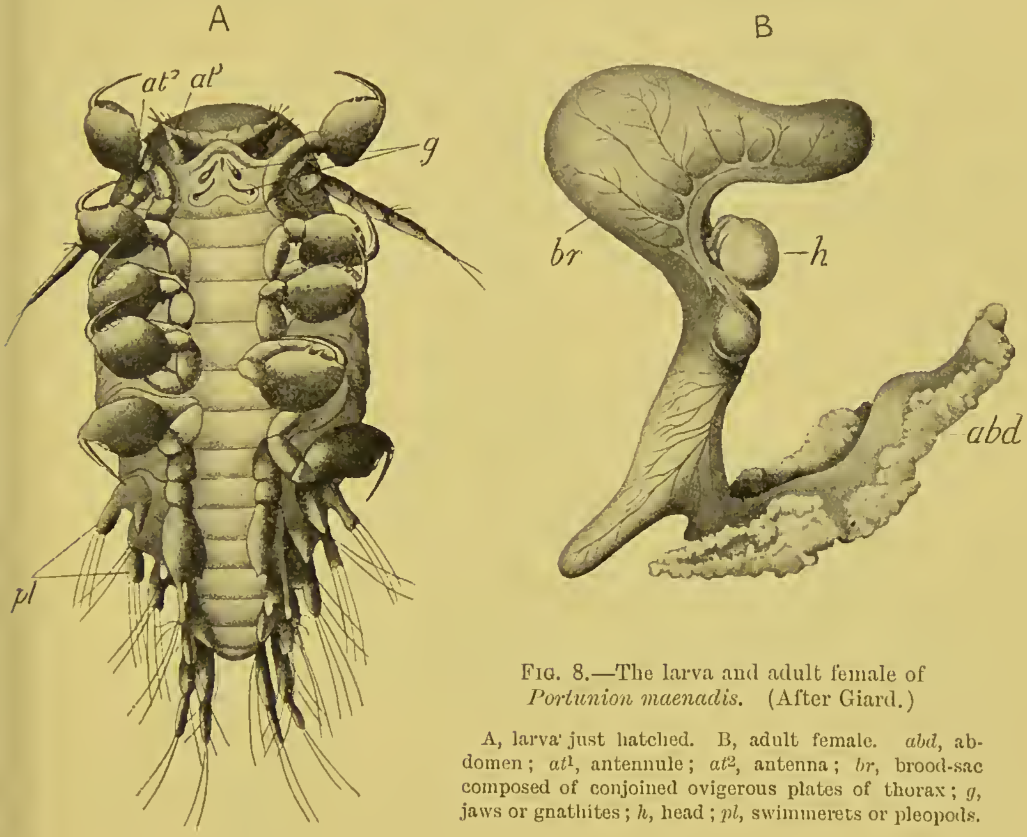

FIG. 8.—The larva and adult female of *Portunion maenadis.* (After Giard.)

A, larva just hatched. B, adult female. *abd*, abdomen; at^1, antennule; at^2, antenna; *br*, brood-sac composed of conjoined ovigerous plates of thorax; *g*, jaws or gnathites; *h*, head; *pl*, swimmerets or pleopods.

If an animal assumes a new habit or mode of life with success, this can only be because a new and abundant food supply is thereby opened up to it. Now Darwin has suggested that a rich food supply is the proximate cause of the arrival of variations. Hence we may provisionally assume that the new food supply upsets the stability of heredity by altering the chemical constitution of the hereditary substance in the germ cells, and so variations in all directions are produced, and those variations are preserved which adapt the organism at the right time to the new mode of life.

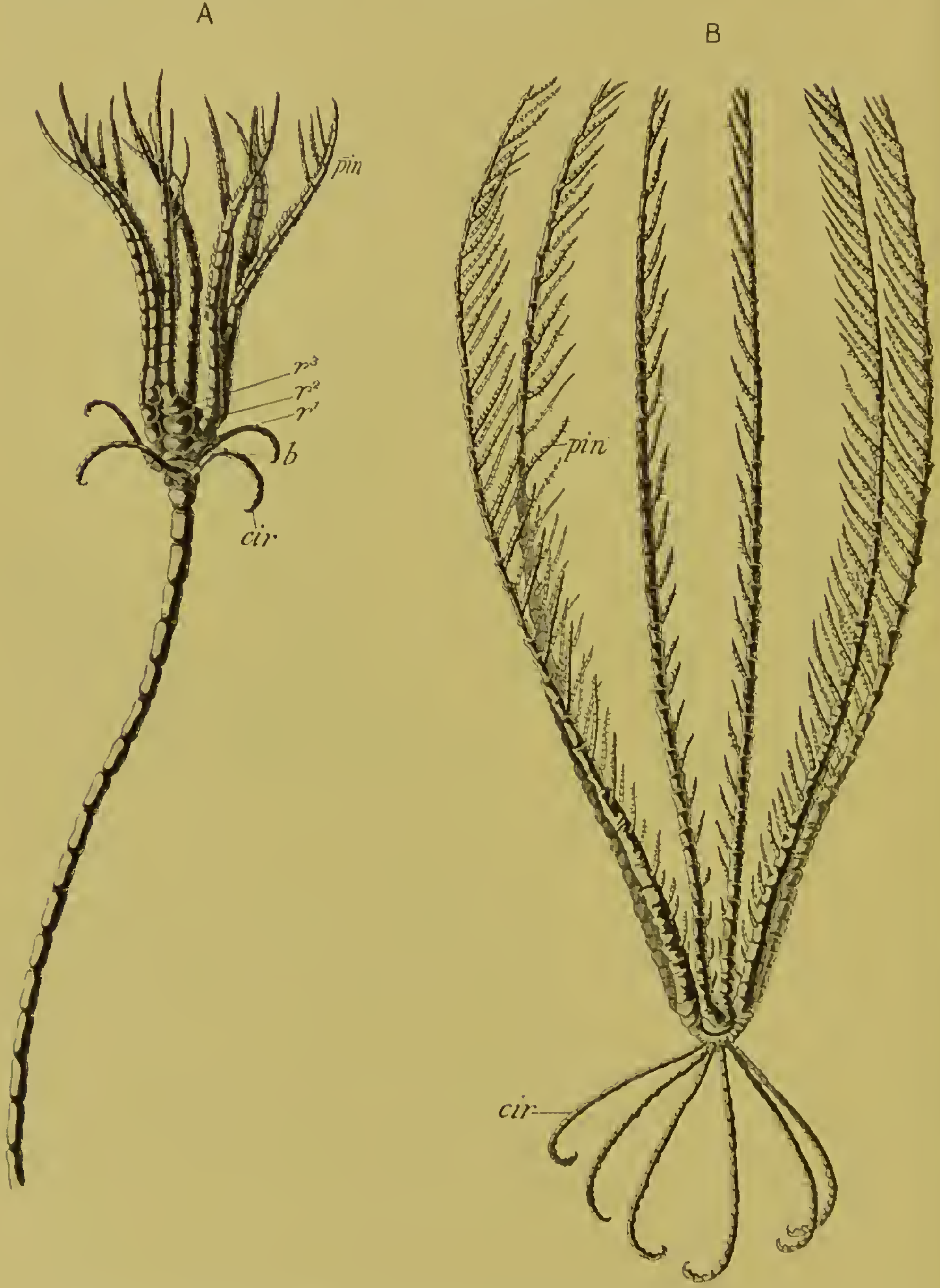

Fig. 9.—The stalked larva and adult form of *Antedon multispina*. (After P. H. Carpenter.) A, larva. B, adult. *b*, Basal plate; *cir*, cirrus; *pin*, pinnule; r^1, first radial plate; r^2, second radial plate; r^3, third radial plate.

If, then, the last stages in developmental history are, so to speak, *the record of the last habits assumed by the species, the main framework of all developmental history must be the condensed record of ancestral experience; for each stage in the development of an animal bears the same relationship to the one which immediately precedes it as the adult stage does to the last larval stage.*

We must now consider some of the influences which modify the ancestral character of developmental history. It is obvious that there is no *a priori* impossibility that the supply of rich food should produce variations which affect earlier stages of the life-history than the adult stage, especially if the alterations produced thereby have a "survival value." Thus we may get secondary adaptations of the larvae to their environment, which are seen to be secondary for the reason that they differ widely from each other within the limits of a group in which the adult structure is constant. When, for instance, within the order of two-winged Flies we find some larvae adapted to living in water, others to living in earth, others in dung, and still others in dead bodies, and a few in living bodies, we cannot regard any of these methods of life as ancestral, and we find that in each case the larva is specially altered to suit it to the special conditions of its existence.

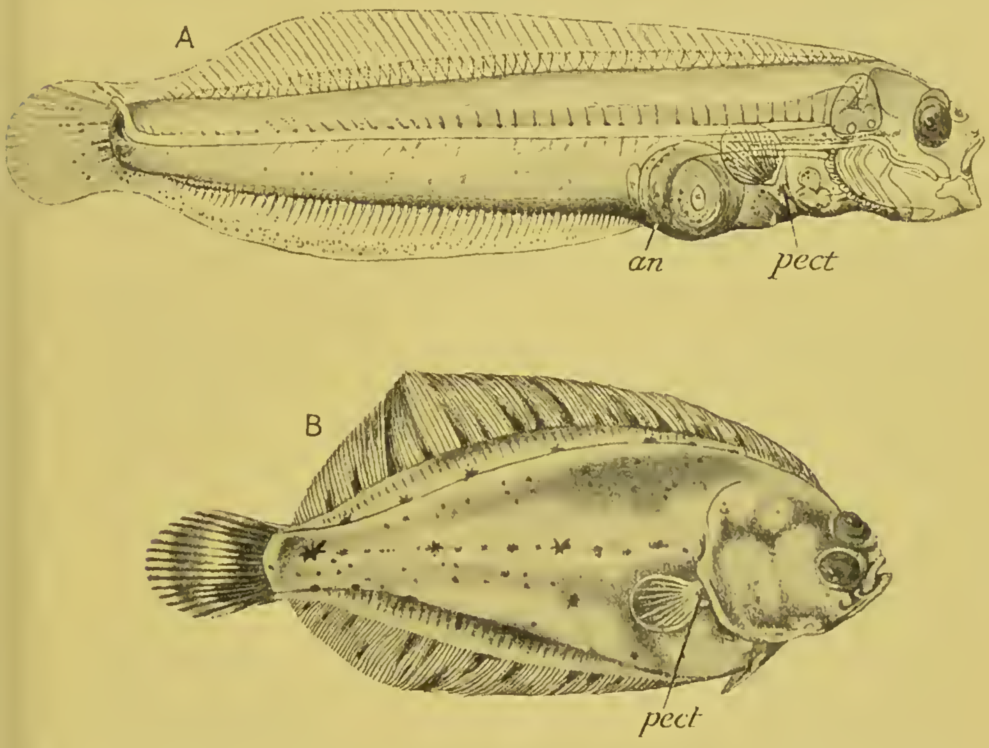

Fig. 10.—The larva and first metamorphosed form of the Plaice (*Pleuronectes platessa*). (After Cole and Johnstone.)

A, larva. B, young Plaice just after metamorphosis. *an*, anus; *pect*, pectoral fin.

The most widespread alteration in the conditions of the larva which is met with is its transformation into an embryo through its retention within the egg-shell or the mother's body. Since, as a race progresses from point to point in evolution, it should, according to the "biogenetic" law, leave behind it a trail of larval stages, each corresponding to a condition of life which had formerly been the adult one, and in each of which the organism would have a distinct method of obtaining its food and a special set of enemies, a very long and complicated life-history should be produced. But the dangers of such a long larval life are very great, therefore a great advantage would be obtained by passing over some of these stages within shelter, and, as was pointed out above, in all life-histories we find an embryonic stage at the beginning.

Now the food necessary for development during the embryonic phase is, in the vast majority of cases, furnished in the form of yolk platelets, embedded in the cytoplasm. This yolk sometimes distends the embryonic cells to enormous proportions; it renders the process of cell-division difficult, and sometimes even impossible. In the ordinary process of cell-division each daughter nucleus becomes at, and immediately after the time of nuclear division, the centre of an attractive force which tends to collect the cytoplasm round it like a ball. In some cases, in consequence of this force, the first products of the division of the egg appear as spheres touching one another only at a point. But this period of activity is succeeded by a period of quiescence, and the centripetal force subsides, so that the cytoplasm of the two daughter cells tends to flow together again, unless a cell membrane has been formed between them in the meantime.

When yolk is present it impedes the action of the centripetal force, apparently by rendering the cytoplasm less viscous; for cytoplasm devoid of yolk behaves like thick honey, whilst that which is loaded with yolk behaves more like a mixture of honey and water. Consequently, in yolky eggs we find that in the first stage of their development they either divide into a few large clumsy cells, or else that cell division is represented by nuclear division only. Further, since the yolk is never uniformly distributed in the ovum, but is usually massed at one side, the first divisions result in the production of cells of unequal sizes or in the production of a cap of cells at one side of the egg, the rest remaining unsegmented. The pole of the surface of the egg which is relatively freer from food-yolk, and where the division into cells first occurs, is termed the **animal pole** of the egg; it is here too that the polar bodies are given off. The opposite pole, where most of the yolk is accumulated, is termed the **vegetative pole.**

Such eggs are termed **meroblastic**, whilst eggs in which the yolk is sufficiently small in amount to allow of the division of the whole are said to be **holoblastic.** Further, whilst in most eggs the yolk is massed at one side **(telolecithal)** (*vide* Fig. 3 A), in a wide range of eggs it is massed at the centre, surrounded by a rind of comparatively yolk-free protoplasm **(centrolecithal)** (Fig. 11). The result of this latter distribution is that a skin of cells is formed over an inert mass of yolk. But the clogging influence of yolk extends far beyond the first stages of development.

The course of development can indeed be roughly divided into three stages:—(1) In the first the zygote becomes divided into a number of embryonic cells or blastomeres; this stage is called **segmentation**; (2) in the second these cells are arranged so as to form the primary organs, the so-called **germ layers,** *i.e.* the skin, and the lining of the gut and of the body cavity; this stage is called the **formation of the layers**; and (3) in the third stage these layers are modified into the larval or permanent organs; this last stage is called **organogeny.**

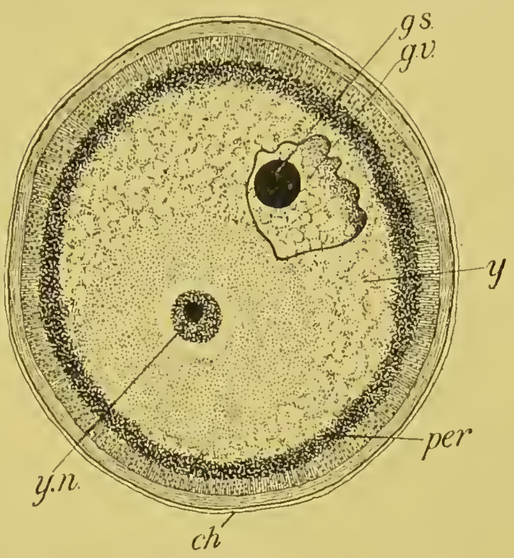

FIG. 11.—Unripe egg of *Limulus polyphemus.* (After Munson.) An example of a centrolecithal egg.

ch, chorion; *g.s,* nucleolus (germinal spot); *g.v,* nucleus (germinal vesicle); *per,* peripheral cytoplasmic area free from yolk; *y,* central area of cytoplasm filled with yolk; *y.n,* yolk nucleus.

Eggs with little or no yolk are termed **alecithal** (Fig. 12). If yolk in the form of refringent globules should be totally absent, reserve stuffs in the shape of masses of chromatin are scattered about through the cytoplasm. In such eggs the building up of organs out of the first cells, or **blastomeres** which result from division, takes place by the simplest processes of unequally rapid growth of different parts, and of folding. Now in the folding of a layer of cells it is essential that the radius of curvature should bear such a relation to the size of the individual cell that the latter should not be deformed. When the layer consists of a few large yolky cells, folding becomes impossible and is replaced by proliferation of new cells at one point in the layer.

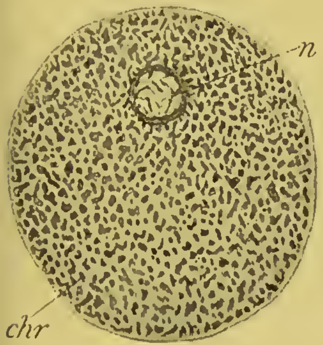

FIG. 12.—The ripe egg of *Strongylocentrotus lividus.* (After Schaxel.) An example of an alecithal egg.

chr, deposits of chromatin scattered through the cytoplasm and acting as reserve material; *n,* nucleus.

Food, as we have seen, is usually supplied to the immature ovum by the sacrifice of the less fortunate oogonia or immature ova. In the case of the common polyp, *Hydra,* the immature egg comes at this stage to resemble an *Amoeba.* But in one or two groups of animals the immature eggs destined to destruction are supplied to the zygote or fertilized ovum to be used as food. This leads to the strangest modifications of the early stages of development. The cells which result from the first divisions of the zygote may actually separate from one another and come together again in such a way as to surround the follicle cells, and this has led to the statement that, in certain cases, the egg dies and the embryo is developed out of follicle cells, but there is apparently no justification for this statement (cf. p. 635).

But an embryonic stage may be, so to speak, intercalated between two larval stages. In the history of the race the change of habits which is recapitulated in the life-history, must have been continuous, for no animal ever suddenly changed from one mode of life to another. Now the dangers incident to larval life and the opportunities of obtaining food, may vary very much, and will be much greater in some stages than others. If in one stage a large store of nourishment can be accumulated, it will be an advantage to the animal to pass quickly over the next stage, which is probably less favourable, and so we may get these intercalated embryonic, or, as they are usually termed, **pupal** stages. During these the animal is sometimes as quiescent as a true embryo, as in most insects ; in others, such as Cirripedia and Holothuroidea, it is active but takes no food.

But there is one outstanding feature about most larvae which strikes the observer, and that is their *extremely small size* compared with that of the adult into which they eventually develop. This reduction in size is in all probability a secondary modification, but it has led to other modifications. An alteration of size produces an alteration in the physiological relations of the organism, and we find that where, from other evidence, we have reason to suspect that the ancestor had a long series of similar organs, the larva may only show one or two ; for all these organs, if reduced to the same scale as that to which the whole body of the animal has been diminished, would become physiologically ineffective. Take, for instance, the gill slits in the larva of *Amphioxus.* These must have a certain minimum size if they are to work, on account of the viscosity of water, and therefore whilst they remain larger in proportion than the other organs of the body, their number becomes diminished, and so where the ancestor had almost certainly two rows of such slits, we find them represented in the larva by one row of slits which occupy the whole ventral surface.

Lastly, it may be remarked that whereas it is true, generally speaking, that the more primitive features an adult exhibits, the more primitive features are found in the larva, yet the change from the larval to the embryonic method of development seems to take place quite independently of the status of the adult, and some animals preserving very primitive features have a development almost completely embryonic, whilst others higher in the scale retain a long larval history.

If it be asked why all animals have not exchanged the larval for the embryonic type of development, considering the advantage which the embryonic phase possesses, from the point of view of the safety of the young organism, it must be pointed out that the larval form of development offers compensating advantages from the point of view of wide dispersal of the species. The balance between these two alternatives seems to have been easily inclined one way or the other.

It is therefore of the essence of **Comparative Embryology** to separate the fundamental ancestral traits of development from the superficial and secondary, and this is the task that has been patiently pursued for the last thirty years. As Sedgwick has pointed out, its results have been highly disappointing, and this has led many to doubt the validity of the ancestral explanation of development. But the reason for this disappointment is largely a human failing which will lead to equal disappointment in any branch of science. This human failing is the ardent desire to settle fundamental questions in a few years. Obviously the most difficult pages of the embryonic record to decipher would be the earliest, for these have suffered most secondary modification, and yet it is precisely over such questions as the first differentiations of the embryo, such as the formation of the primary tissues or so-called "layers" of ectoderm, endoderm, and mesoderm, that most of the divergences of opinion have arisen.

When we allow the mind to contemplate the vast profusion of living species at present in the world, each with its own peculiar life-history, and then reflect how few are at all known, we can see at once how small a clearing we have made in the forest of comparative embryology, and how premature it is to abandon the hope of finding a law underlying the likenesses and unlikenesses of the various modes of development. Where, as in the case of Vertebrata, the knowledge is more complete than in the case of other groups, the recapitulation of the structure of the lower members of the group in the young stages of the higher, is so plain as to be obvious to all. When the knowledge of other groups becomes equally complete the same thing will be obvious there also.

Those who have abandoned Comparative Embryology for Experimental Embryology have set themselves the task of finding out the mechanism of the transformation of the apparently formless egg into the differentiated adult. But here again the impatience with delay, the determination to arrive at "basal" principles at once, will prepare disappointment for the workers in this branch also.

Thus we find, as already pointed out, that whilst Driesch arrives at the conclusion that each kind of life-history owes its peculiarities to a non-material entelechy—but leaves the resemblances between the life-histories utterly unexplained, Herbst arrives at the conclusion that in each stage of development a substance is found which acts as a "stimulus" to cause the development to the next stage, while Loeb on the other hand maintains that until the conception of "stimulus" is utterly abandoned no real progress with the subject will be made. Here we have divergences as great as those which exist between any upholders of rival phylogenetic theories.

The real truth is that Experimental Embryology is an adjunct and not an alternative to Comparative Embryology. It is a new and refined instrument of dissection: instead, for instance, of separating the blastomeres of a segmented egg by optical differences they are actually separated and their values tested by their powers of development. But the difference between the Echinoderm egg where any of the first eight blastomeres will develop into a whole larva, and the Annelid egg where the loss of a blastomere means the loss of a portion of the larva, still requires for its explanation the principle of affinity, that is to say that the ultimate explanation of the specific peculiarities of development is found in the chemical nature of the hereditary substance.

So, before the future student of embryology stretches an almost limitless field of research. We must ultimately find out not only *how* the chemical quality of the germ-plasm determines the growth of the formless egg into the highly complex adult, but we must also find out how this chemical quality can be altered, so that variations can occur and evolution can take place, and this is the root-problem of biology.

In order to make any attempt to solve this root problem we must, however, be able to control the whole life-cycle of the animal experimented on, and this is precisely where the work of Loeb, Herbst, and Driesch breaks down. All these workers have chosen as experimental objects the eggs of Echinoderms. These eggs are produced in enormous numbers and are easy to rear through the first stages of their development, but to reach even the adult form—to say nothing of the adult dimensions—they have to pass through a prolonged larval life during which there is an enormous mortality, and even when this metamorphoses into the adult shape is successfully accomplished they have less than the millionth of the bulk of the fully ripe sexual form. Under the most favourable circumstances a year or two must elapse before they could produce germs and, therefore, before it could be possible to say whether the experiments had really altered the hereditary potentialities, or whether the distorted larva is merely the resultant of the new force applied and of the unaltered hereditary potentiality of the germ. The method of rearing these larvae until they attain sexual maturity has now been elucidated, but only a small proportion of the fertilized eggs can so far be reared; and none of the workers mentioned above have attempted to rear the larvae beyond the earliest stages of their development.

We pass now to consider the special embryology of the different groups of Invertebrata. In every case we shall, so far as possible, give examples of the fundamental laws of development laid down in this introductory chapter, and we shall indicate also what solid results have been gained from experiments performed on the developing eggs of animals belonging to each group.

The group Protozoa are excluded, not because they do not in many cases exhibit a development showing larval and embryonic stages, but because in most cases it is not easy to determine what corresponds to the adult stage in Protozoa, and their life-histories are too imperfectly known for profitable comparison.

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CHAPTER II

PRACTICAL HINTS

The object of this book is not merely to lay before the reader the best ascertained results of embryology, it is also designed to indicate the directions in which further research may be most advantageously prosecuted, and to suggest reliable methods of pursuing such researches. Incidentally defects in the methods employed by some investigators, and the possible bearing of these defects on their results, will be pointed out.

In the present chapter some general instruction will be given on methods of procedure which are applicable to all, or nearly all classes of embryo, while special methods will be described when each separate phylum is described.

When one endeavours to work out the life-history of an animal the first step is to observe the larvae or embryos in the living state. In many cases a large number of points can only be made out in the living embryo, since the tissues are then in their natural state of turgescence, and living protoplasm is relatively transparent. The next step is to preserve or *fix* the embryos, dehydrate and clear them and mount them whole.

Fixing or preservation consists in adding some reagent to the specimen to be preserved which will form a stable and more or less solid compound with the protoplasm of the organism. This compound enables the form of the organism to be retained during the process of dehydration, and the macerating and deforming effects of the diffusion currents produced in this process to be resisted. Dehydration, is the removal of the water by successive immersion of the object in different grades of alcohol; clearing, is infiltration of the tissues by an oil like oil of cloves, cedar oil, etc., which renders them transparent.

Now the reagent which forms the strongest compound with protoplasm and preserves in it the nearest resemblance to its living condition is the solution of **osmium tetroxide** in water, usually erroneously called **osmic acid.** For effective fixation a solution of at least ·25 per cent must be used. "Osmic acid" has two disadvantages, it produces a very black stain which consists of the metal osmium, and it is apt to render the tissues brittle. Further, if applied to objects of any size osmic acid forms a crust of hardened imperviable protoplasm which prevents the penetration of the reagent into the interior. It is, therefore, a reagent eminently suited for the preservation of minute larvae and the permeable tissues of calcareous sponges.

For the denser tissues of siliceous sponges other reagents would be more suitable; such, for instance, as a mixture of 3 parts concentrated solution of **corrosive sublimate** in water and 1 part of **glacial acetic acid.** This is one of the best and most universally employed preservatives: many investigators use, however, a smaller proportion of acetic acid (often as low as 5 per cent) than that just mentioned; it is to be remembered that acid reagents are unsuitable for calcareous sponges and for other organisms which contain much calcareous matter, because the evolution of carbonic acid gas dissolves the calcareous matter, and so causes the formation of blebs in the tissues and of artificial rents and cavities which have no counterpart in the living animal. When it is desired to decalcify, this is best accomplished when the organism is in strong alcohol. If a drop or two of nitric acid be added to a small bottle (of two fluid ounces) full of strong alcohol and well shaken, a solution is produced which will decalcify so slowly that the resulting gas is at once dissolved and never forms bubbles.

A different method of decalcifying organisms which have been preserved in osmium tetroxide may be mentioned here. If, after being blackened by immersion in the solution and then rinsed in clean water, the specimens be immersed in **Müller's fluid**, not only will the calcareous matter be slowly removed but also the excess of metallic osmium, and the tissues will be rendered less brittle. Müller's fluid is a mixture of bichromate of potash, which contains unsaturated chromic acid and sulphate of sodium. **Flemming's fluid**, which is a very favourite preserving medium, is really an attempt to combine the advantages of osmium tetroxide and chromic acid, for it is a mixture of these two fluids with acetic acid. It is an excellent preservative, but is intensely acid and open to the same objections as other acid reagents. The same remarks apply to **Hermann's fluid**, which is a mixture like Flemming's fluid, in which acid platinic chloride replaces the chromic acid.

When it is desired to make whole mounts of minute forms it will generally be found that osmium tetroxide, corrosive sublimate, etc., render them too opaque. Strong **formalin**—that is a 40 per cent solution of the gaseous formic aldehyde in water—is a splendid reagent for this purpose. It kills small larvae instantaneously, without any shrinkage. It is apt, however, to become acid by the oxidation of the aldehyde into formic acid; it is therefore advisable to carefully neutralize the solution before employing it. Further, the compound which it forms with protoplasm is soluble in water. Therefore, after a few minutes' sojourn in the formalin solution, the specimens must be instantly transferred to absolute alcohol, and in this they must be stained. Eosin or methyl green dissolved in absolute alcohol are very good stains. The transference to oil of cloves must be made by adding this substance drop by drop to the absolute alcohol at intervals of an hour or so for several days. After a sojourn in the pure oil the specimen is placed in the concavity of a hollowed slide and suddenly covered with thick solution of Canada balsam in xylol. The oil of cloves flies to the periphery of the balsam owing to surface tension and may be removed by blotting-paper.

When all the information possible has been gleaned from whole mounts of embryos and larvae the next step is to cut them into series of sections arranged in order, but for this purpose they must be embedded in a block of paraffin so that the sections when cut by the microtome will be parallel to a known direction. To accomplish this placing, or **orientation**, as it is called, in the case of minute larvae is a matter of great difficulty, and unless the sections are cut in the right direction they are very difficult to interpret. The best way to overcome this difficulty is to embed the specimens in celloidin before embedding in paraffin. The solution of celloidin used for embedding vertebrate tissues, consisting of celloidin dissolved in a mixture of equal parts of absolute alcohol and ether, is not suitable for delicate larvae because too violent diffusion currents are produced in the process of changing from alcohol to the celloidin solution. If, however, the celloidin be dissolved in a mixture of four parts of absolute alcohol and one part of ether, then such currents are avoided. It is well to have three grades of this solution, one saturated, one made by diluting the saturated solution with an equal bulk of the solvent, and one by diluting it with two volumes of the solvent. The objects, if they are small, should remain in each grade for about one day. Then the thick solution with its contained embryos is poured into chloroform and the celloidin hardens to a cheesy consistence. After an hour's sojourn in this fluid a piece of celloidin containing the embryo can be cut out and embedded in paraffin.

The embedding may be done in one of two ways.

(1) The piece of celloidin containing the object is placed in absolute alcohol, to remove any trace of moisture, and then immediately transferred to fresh chloroform to which fragments of clean paraffin are added. If the whole be heated to 60° for an hour all the chloroform will have evaporated and the object can now be poured, together with some of the paraffin, into a mould and allowed to cool. Before transferring to the mixture of chloroform and paraffin, the object can be studied under the lower power of the microscope and the celloidin shaped so as to direct the orientation of the block of paraffin.

(2) The object and its surrounding celloidin may be transferred to cedar oil. If this be warmed for half an hour (by being placed on the top of the thermostat) all traces of moisture will be absorbed, and the cedar oil will render the celloidin absolutely transparent, so that the object can be examined as if it were mounted in oil of

cloves. The celloidin should then be cut as before so as to indicate the position of the object, and the latter, in its celloidin block, should be transferred to a mixture of cedar oil and hard paraffin and heated to 56° for fifteen minutes, and then for fifteen minutes to a bath of pure hard paraffin.

This second method has the disadvantage of rendering the embryonic tissues rather brittle, but one great advantage of embedding in celloidin is that the tissues of the embryo become penetrated while cold by a substance which hardens and gives them support, before they are subjected to the ordeal of the hot paraffin bath, which has a tendency to cause shrinkage.

When the sections are cut they are best mounted by first smearing the slide with Mayer's glycerine and albumen fixative, then laying the sections upon it and adding a layer of water heated to 60°; the hot water cools at once to about 45°, and this heat will flatten out the sections without melting the paraffin. The water is drained off and the slide dried on the top of the thermostat for forty minutes. Then the paraffin can be melted off and washed off in xylol. It should then be immersed in oil of cloves for one minute, which softens and dissolves the celloidin. Then it should be placed, not in absolute alcohol, but in 90 per cent alcohol, which washes off the oil of cloves and at the same time removes the glycerine from the glycerine and albumen fixative; it finally coagulates the latter and also hardens the semifluid celloidin, so that it forms an additional fixative for securing the adhesion of the sections to the slide. Another method is to transfer the slide from pure xylol to a mixture of equal parts of xylol and absolute alcohol. The absolute alcohol coagulates the albumen and removes the glycerine. The slide can then be transferred to 90 per cent alcohol and thereafter to alcohol of lower grades. Sections thus fixed will stand any further treatment without becoming loose.

Mayer's fixative is made by mixing white of egg strained through muslin with an equal volume of glycerine. A few drops of thymol are added to prevent the decomposition of the albumen.

In staining objects which have been preserved in osmium tetroxide it is often found that the black deposit of metallic osmium in the tissues prevents the stain from taking effect. The best general stain is Grenacher's (sometimes called Delafield's) haematoxylin. This is best used in a solution made by diluting the concentrated stain in three or four times its bulk of distilled water. This solution should be filtered before being employed. If the sections be previously immersed in a solution of borax-carmine in 70 per cent alcohol it will be found that they can remain in it for 24 hours without absorbing any stain, but if then they be transferred to the solution of haematoxylin described above, they stain rapidly and well. Excess of stain is removed by immersing the sections in a solution of acid alcohol made by adding two drops of strong hydrochloric acid to 100 c.c. of 70 per cent alcohol. If the sections be examined from time to time under a microscope as the stain is being removed, a point will be detected at which the whole section takes on a reddish colour, and the nuclei stand out prominently. When this is observed the sections should be washed free from the acid alcohol by immersing them in 70 per cent alcohol. They should then be held inverted for a few moments over the mouth of a bottle of strong ammonia, the escaping fumes from which neutralize the last traces of acid, and the sections, now of a beautiful blue colour, may be dehydrated and mounted. The different tissues yield up their stain in different degrees, and a beautiful differentiation is effected by the different tints of blue.

If a double stain be desired it can be effected by finally dehydrating the sections in absolute alcohol to which eosin has been added, but if they remain too long in this solution all the haematoxylin will be washed out.

The methods described in this chapter are general methods applicable to all classes of embryos; special methods will be described in the chapters dealing with special groups.

CHAPTER III

PORIFERA

Classification adopted—

- **Calcarea**
  - **Homocoela (= Asconidae)**
  - **Heterocoela**
    - **Syconidae**
    - **Leuconidae**
- **Triaxonia (= Hexactinellida)**
- **Demospongiae**

THE group Porifera or Sponges stand apart from all the rest of the Metazoa, and their embryology is consequently of very great interest. We may suggest as a form for practical study the development of the Calcareous sponge *Grantia compressa.*

This sponge, distinguished by its flattened shape, is a common denizen of the British coasts, and its embryology is being worked out by Professor A. Dendy. Allied species occur on the coast of North America; and the course of its development, so far as determined, so closely resembles that of the Mediterranean species, *Sycandra raphanus*—the subject of Schulze's classic research (1875)—that the latter may be taken to represent that of *Grantia* and of Calcarea generally.

SYCANDRA RAPHANUS

The eggs are found embedded in the jelly which forms the substance of the wall of the sponge, intervening as it does between the cells forming the dermal membrane and those lining the paragaster and the extensions of this latter cavity into the flagellate chambers. The spermatozoa occupy a corresponding position in the male. When ripe, they bore their way through into the flagellate chambers, and are discharged by the osculum. They swarm in the surrounding water, and, coming under the influence of the inhalent currents of the female, they penetrate through its pores and thence find their way to the eggs, which are thus fertilized *in situ.*

The fertilized egg undergoes the first stages of its development in the maternal tissues. It is found to be contained in a cavity lined by a definite layer of dermal cells. This cavity is wedged in between the layer of dermal spicules and a flagellated chamber. As it enlarges to suit the size of the growing embryo, it encroaches on the cavity of the flagellated chamber, since the layer of dermal spicules is unyielding. (Dendy, 1889.)

The first stages of development must therefore be studied in transverse sections of the adult.

When the larvae emerge they must be encouraged to settle on some convenient portable object. If it is desired simply to make whole mounts, the bottom of the vessel in which the parents are contained is strewn with coverslips, and these are removed when the young sponges have attached themselves to them, and immersed in 1 per cent solution of osmic acid till fixation is effected, then stained in picro-carmine and mounted whole. If it is desired to cut sections of the larvae, the coverslips must be covered with a layer of paraffin wax or photoxylin, which can be scraped off and the larvae thus removed, when they can be dealt with by the methods described in the previous chapter.

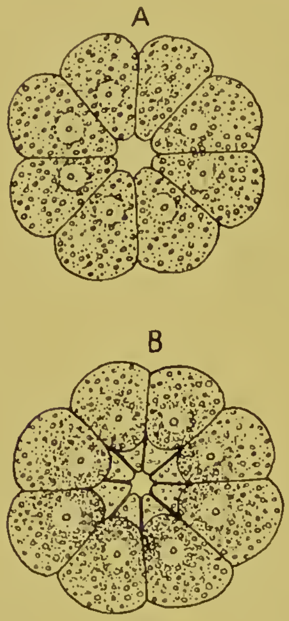

Fig. 13.—Two stages in the segmentation of the egg of *Sycandra raphanus*. (After Schulze.)

A, 8-cell stage in which all the blastomeres are in one tier with a central aperture. B, 16-cell stage blastomeres arranged in two tiers of eight each round a central aperture.

The egg divides into two, four, and eight blastomeres, which are arranged in one plane, and, from the 4-cell stage, they surround a central cavity open at both ends, which owes its existence to their mutual separation (Fig. 13). This stage is followed by a division of all the cells into two tiers, so that sixteen cells are formed in two rows, and then each of these rows is subdivided into two further rows, and so we reach the 32-cell stage. Divisions now follow one another in the individual cells somewhat irregularly, and thus an oval vesicle is constituted, which may be termed the **blastula,** one pole of which is rounded and one flattened, whilst inside it there is a cavity which is a development of the cavity formed by the separation of the first segments of the egg, and which is termed the **blastocoele.**

One opening of this cavity to the exterior, that at the pointed end, is by this time closed, but that on the flattened "basal" surface persists for some time, though it too eventually closes. The cells immediately surrounding this latter pore are distinguished from the rest by becoming extremely granular. The granular cells increase by division to thirty-two, whilst the remaining cells become extremely long and columnar, and each develops a flagellum. The columnar half of the embryo is pressed against the wall of the yielding chamber, but the granular cells encounter the resistance of the spicules, and therefore the embryo becomes hat-shaped. A few small cells are found in the segmentation cavity. Dendy (1889) thinks that these cells, which may be termed **mesenchyme**, have been budded from the flagellated cells, but this is not certain. The granular cells now proliferate rapidly, especially in the centre, and form a thick mass which becomes invaginated into the blastocoele.

The embryo is now ready for birth. By the activity of its flagella it bores its way into the adjacent flagellated chamber of the mother, and then escapes through the osculum. During this process the blastocoele seems to absorb water, the invaginated cells are exserted, and thus the free-swimming larvae acquire an oval form; but the cells forming one half of the wall of this vesicle are granular and rounded, whilst those forming the other half carry long flagella, and possess, in addition, a bright red pigment. The interior is half-filled up with granular cells. Such a larva is termed an **amphiblastula**, and, as we shall see, this type recurs in all families of sponges (Fig. 17).

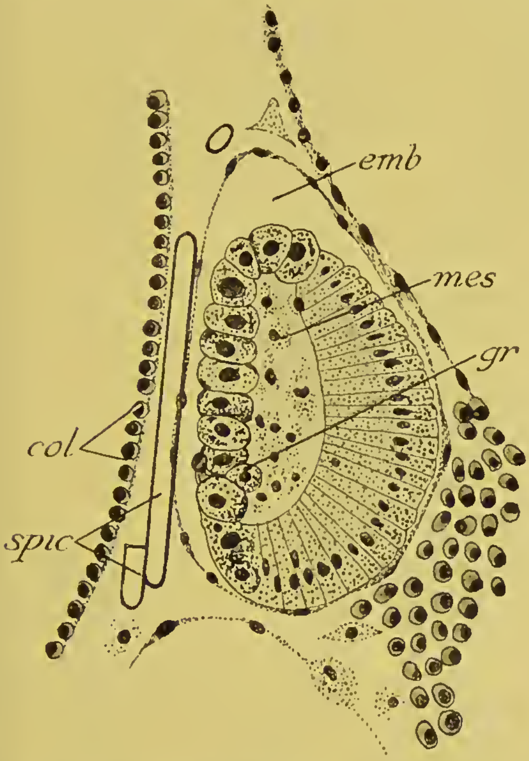

FIG. 14.—View of the embryo of *Grantia labyrinthica* in the blastula stage lying in the embryonic chamber of the mother. (After Dendy.)

col, collared cells lining a maternal flagellated chamber; *emb*, embryonic chamber; *gr*, granular cells of the embryo; *mes*, cells, so-called mesenchyme budded into the blastocoele; *spic*, maternal spicules.

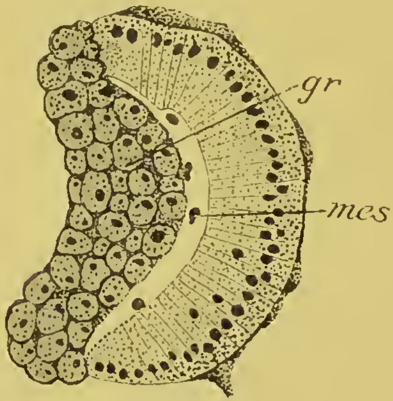

FIG. 15.—View of the embryo of *Grantia labyrinthica* in a later stage of development than that represented in Fig. 14. (After Dendy.)

Letters as in Fig. 14.

After swimming for a day or two the amphiblastula comes to rest on the surface of a smooth stone, its ciliated half which preceded the other whilst the larva was moving, being directed downwards. Within a few minutes the larva has undergone an entire change of shape. Its anterior end flattens out; the ciliated cells which con-

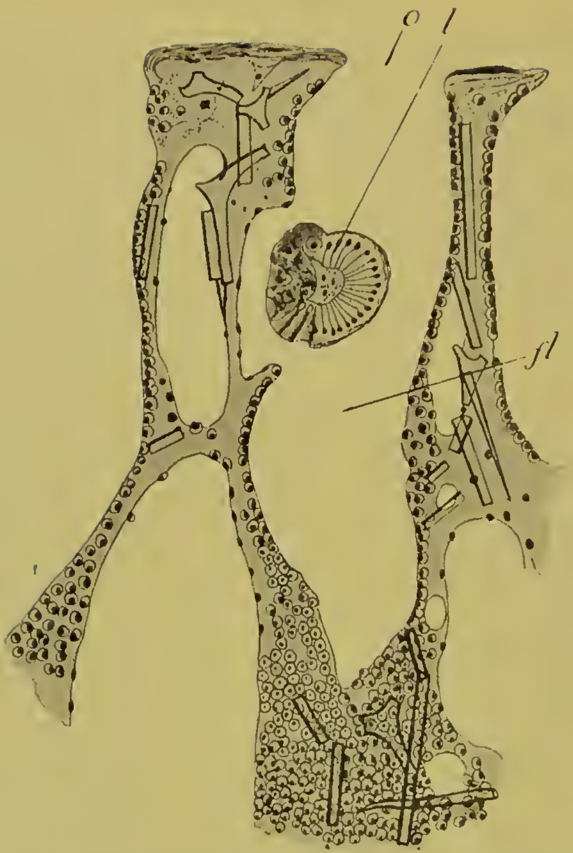

Fig. 16.—Section of a portion of *Grantia labyrinthica*. (After Dendy.)

Showing the escape of the larva from the tissues of the mother sponge into the flagellated chamber of the mother; *fl*, flagellated chamber; *l*, escaping larva; *o*, opening of flagellated chamber into central cavity (paragaster) of sponge.

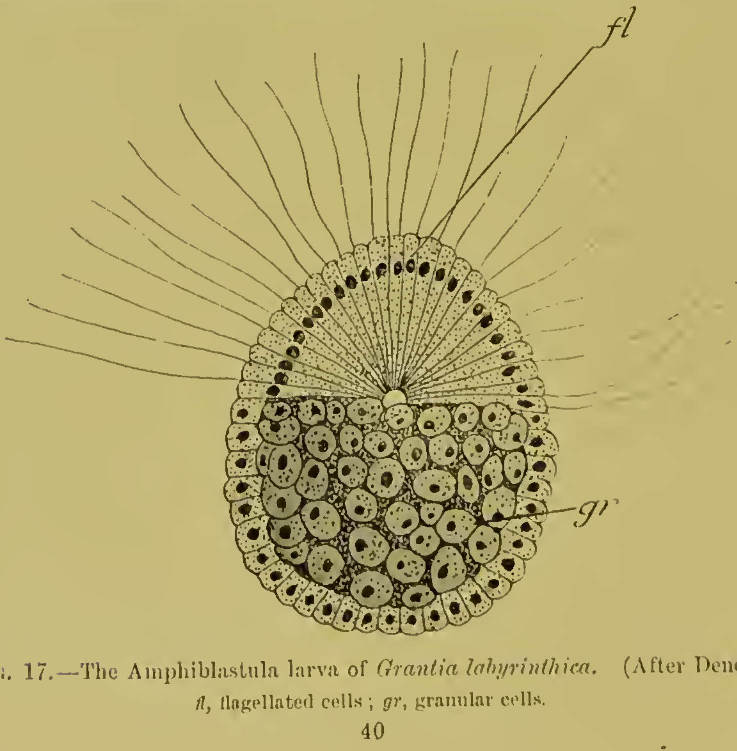

Fig. 17.—The Amphiblastula larva of *Grantia labyrinthica*. (After Dendy.)

fl, flagellated cells; *gr*, granular cells.

stitute it become invaginated into the posterior half, and the blastocoele is thus reduced to a mere slit. The cells forming the edge of the cavity of invagination are granular, and when the ciliated cells become invaginated, these granular cells extend inwards along the substratum, and floor the cavity of the invagination (Fig. 18); they also extend outwards in irregular tongue-like pro-

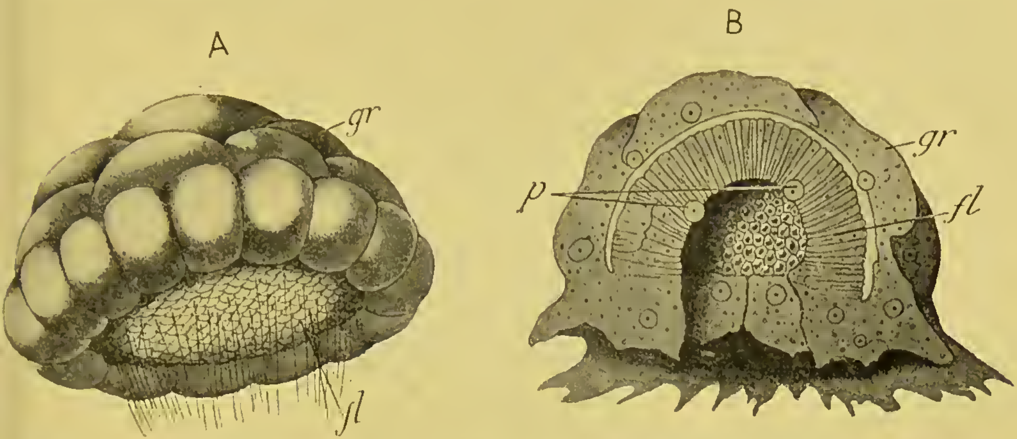

FIG. 18.—Two stages in the fixation of the larva of *Sycandra raphanus*. (After Schulze.)

A, the flagellated cells are just retreating into the interior. B, the larva has assumed the form of a hemispherical cup and is attached by amoeboid processes of the outer granular layer. Seen in optical section. *fl*, flagellated cells; *gr*, granular cells; *p*, porocytes (?).

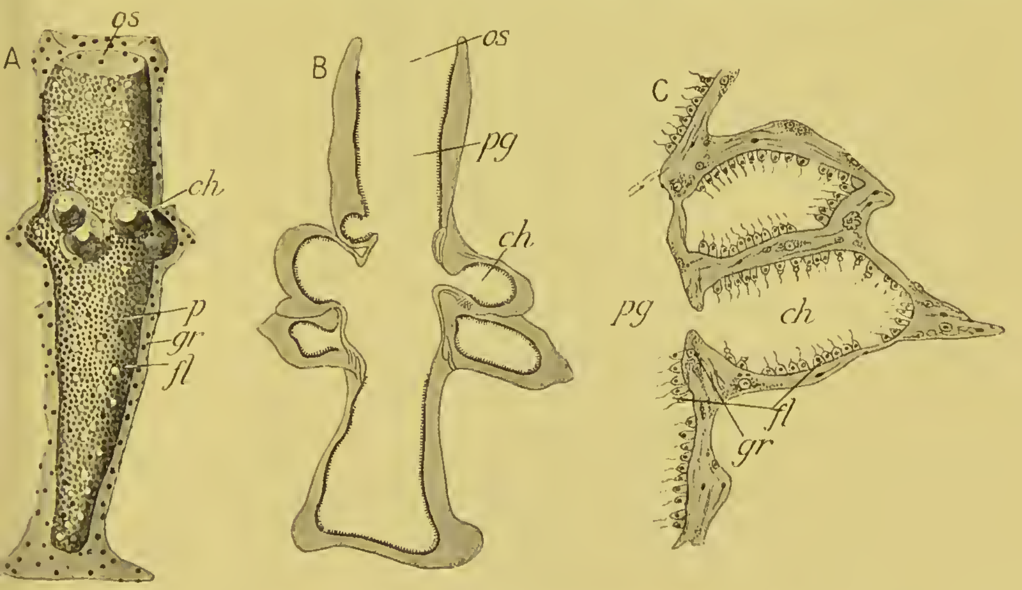

FIG. 19.—An early stage in the metamorphosis of the Ascon stage of *Sycandra raphanus* into the adult. (After Maas.)

A, external view of young sponge. B, diagrammatic longitudinal section of the same to show the gradual displacement of collar cells by granular cells. C, a small portion of such a section further enlarged. *ch*, radial flagellated chamber; *fl*, flagellated cells; *gr*, granular cells (in C the reference line points to a spot where the granular cells are migrating inwards); *os*, osculum; *p*, inhalent pore; *pg*, paragaster.

cesses and adhere to the substratum; the process of attachment of the larva to the substratum is known as **fixation.**

The larva is thus converted into a closed cylinder, the wall of

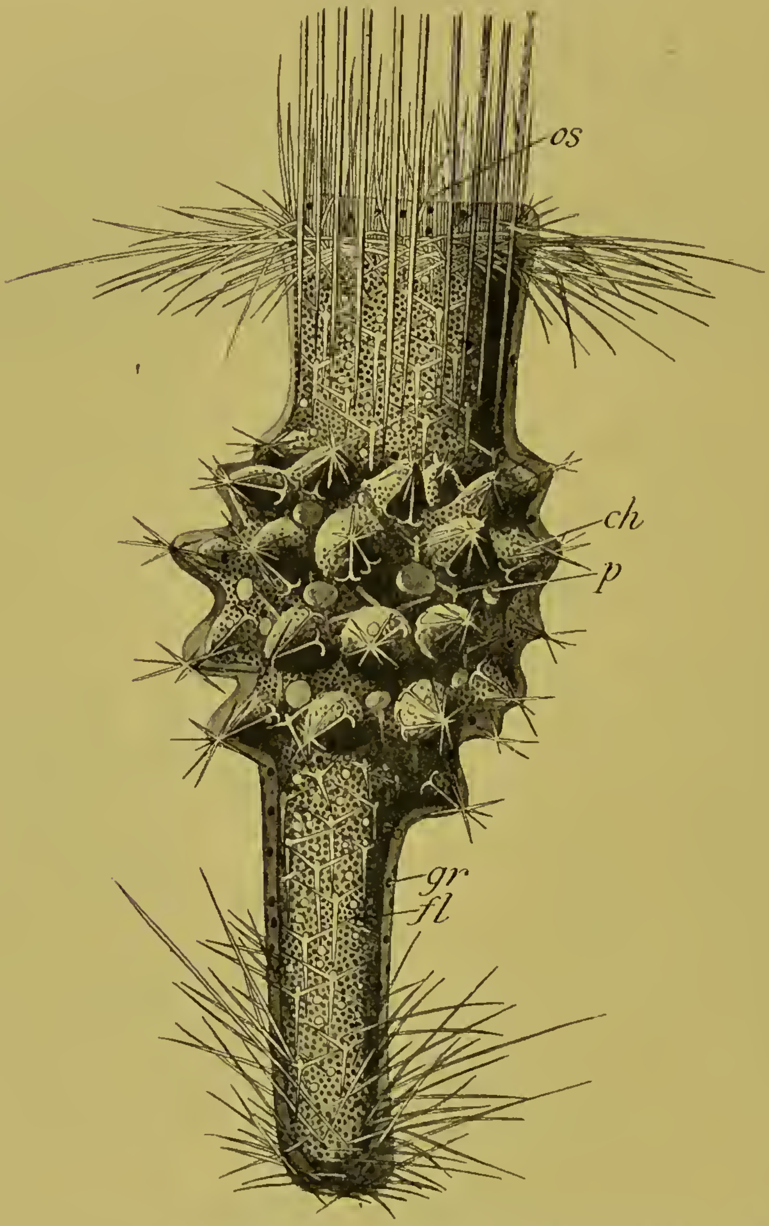

Fig. 20.—A late stage in the metamorphosis of the Ascon stage of *Sycandra raphanus* into the adult condition. (After Maas.)

Letters as in Fig. 19.

which consists of an outer layer of flattened cells, and an inner layer composed of ciliated cells, on each of which the collar soon makes its appearance. This collar is characteristic of the cells lining the flagellated chambers in all Porifera. Between the two layers a layer of jelly makes its appearance, which is the real stiffening element in the sponge-wall.

The formation of **inhalent pores** is now begun. Individual cells of the outer or dermal layer extend inwards through the jelly, and press asunder adjacent cells of the inner or gastral layer. These cells then become hollowed out, converted into drain-pipes, as one might term it, and the action of the flagella draws in water through them. Other cells migrate from the outer layer into the jelly, and form the characteristic calcareous needles or spicules. The first type of cells are called **porocytes,** the second **scleroblasts.** After the pores have been acting for some time, an exhalent opening is formed at the distal end of the cylinder. The formation of this **osculum** seems to be due in part to the hydrostatic pressure caused by the action of the pores.

The tiny sponge is now quite comparable to the type of adult sponge exemplified by the genus *Leucosolenia.* Its transformation into the adult Sycon is an affair of slow growth, and the process has not been observed in this *Grantia,* but there is no reason to doubt that it is essentially similar to what occurs in *Sycandra raphanus,* in which it has been described by Maas (1900).

In *Sycandra,* pouches grow out horizontally from the cylinder which forms the body of the young sponge; they are formed gradually, not all at once. As the pouches are formed the flagellated cells are taken up into them, and the dermal cells migrate inwards from the outside, pressing the flagellated cells asunder, and constitute the epithelium lining the central cavity of the sponge or "**paragaster.**" The interspaces between the openings of the horizontal pouches, that is to say, the niches left between the outer surfaces of these pouches, constitute the **inhalent system of canals.** In this sponge the reproductive cells seem also to be formed from the dermal layer; in their undifferentiated form they are full of yolk, and are known as **archaeocytes.**

OTHER SPONGES

To Maas (1898) we owe the demonstration that all sponge larvae are modifications of the type just described. Of the development of the Hexaetinellida nothing is known; larvae, it is true, have been observed which seem to originate from unfertilized eggs, and which resemble the larvae of other siliceous sponges, but their history has not been followed.

When we turn to the Demospongiae in which the spicules are arranged in cords, and which constitute the vast majority of sponges, we can trace a complete series from a development more primitive than that of *Grantia* to the most modified form. Beginning with *Oscarella,* which, although devoid of a skeleton, has its affinities with the Demospongiae, Maas shows that the embryo is hatched as an oval blastula, consisting of a uniform layer of flagellated cells. During the course of its free life as a larva, the cells of the posterior half lose their flagella and become granular, so that the blastula is thus converted into an amphiblastula. The amphiblastula fixes itself and undergoes a metamorphosis like that of *Grantia*, but the resulting sponge, the "**Rhagon,**" is conical not cylindrical, and the flagellated chambers are produced as hemispherical pouches of the inner layer (Fig. 21).

In the development of the Tetractinellid *Plakina*, Maas (1909) describes the larva as beginning its free life as a blastula, since the cells constituting its wall are at first *all* slender and ciliated, but the blastocoele contains a few rounded granular cells, termed **archaeocytes,** which seem to be the mother cells of the germ cells. The posterior half becomes granular by the alteration of the cells, which lose their cilia, but cells which are not to be confused with the archaeocytes are also budded from this half into the interior (Fig. 23). Fixation and metamorphosis occur as usual, but the resulting sponge has the form of a shorter cylinder than is the case with either *Grantia* or *Oscarella.* By downgrowths of dermal cells, the interior flagellated cells become divided into groups, which, although at first they retain a portion of the lumen of the sponge, eventually become solid; from these solid masses the spherical flagellated chambers are formed later (Fig. 22).

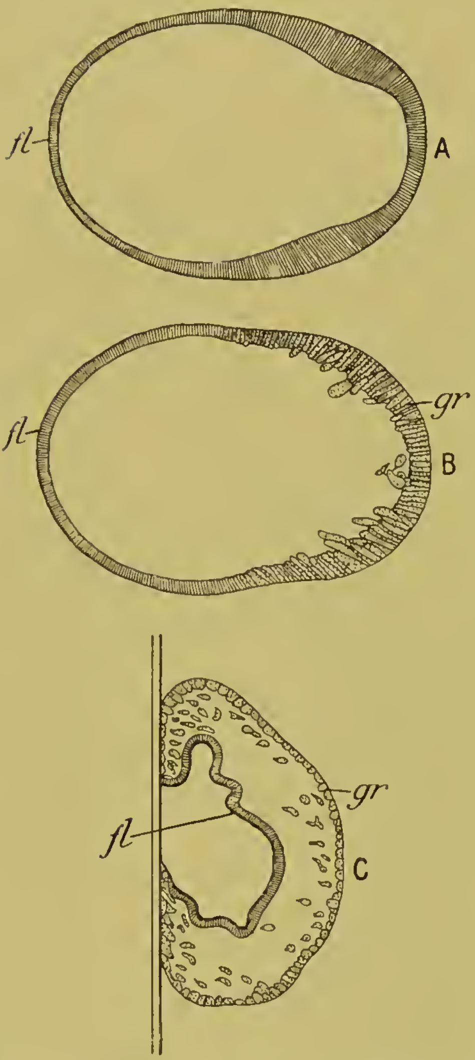

Fig. 21.—Longitudinal sections through the free-swimming larva of *Oscarella lobularis* in two stages of its development and its fixation. (After Maas.)

A, early larva. B, larva in which posterior cells are becoming granular. C, Rhagon shortly after fixation. Letters as in two previous figures.

Finally, in the siliceous sponge, *Esperia* (Maas, 1892), the larva is hatched as an amphiblastula, but the flagellated cells cover four-fifths of the surface, and the granular cells form a solid plug projecting into the interior of the blastocoele and contain a sheaf of siliceous spicules, ready for distribution throughout the tissues of the young sponge as soon as fixation has occurred. Stretching across the blastocoele are branched cells reminding us of the mesenchyme cells of the larva of *Grantia*.

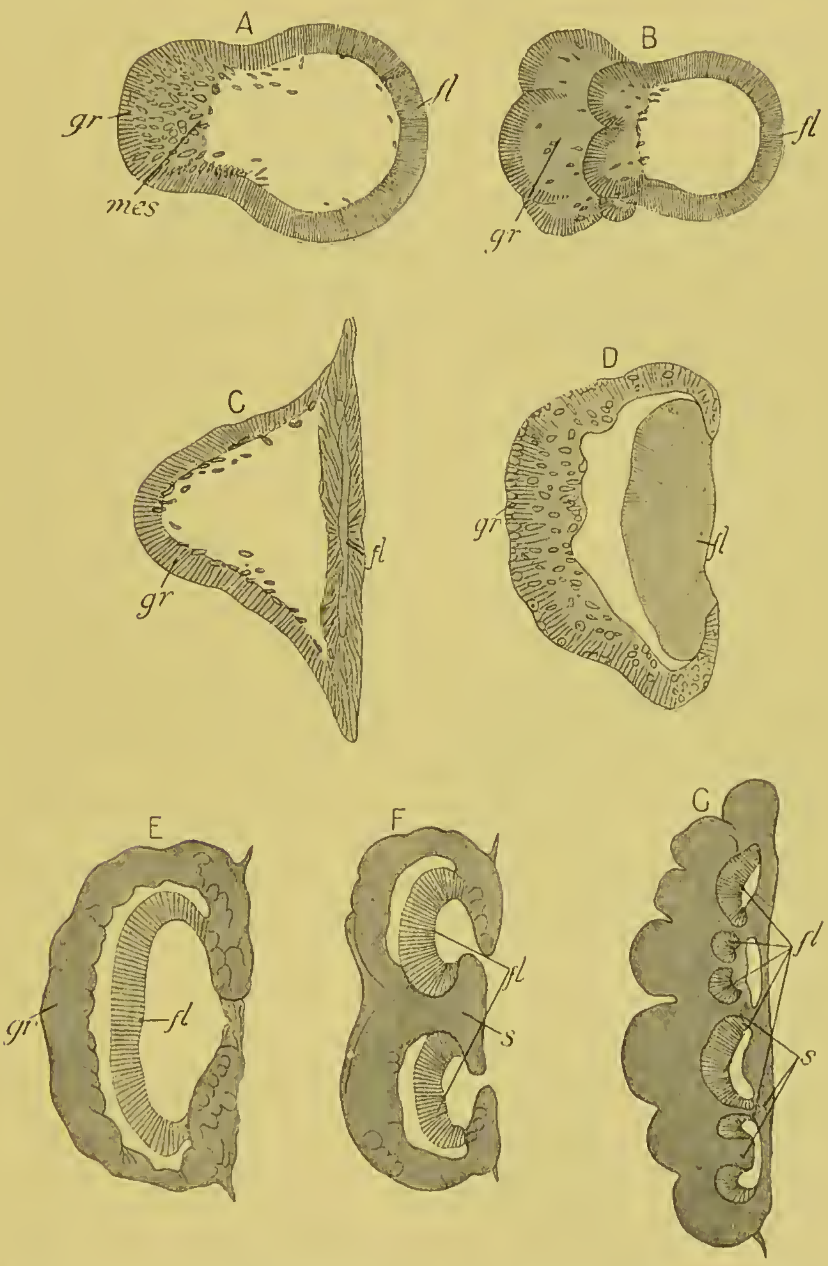

FIG. 22.—Seven stages in the metamorphosis and fixation of the larva and growth of the young sponge of *Plakina monolopha*. (After Maas.)

A, larva in amphiblastula stage; granular cells budding off cells into interior. B, larva about to fix itself. C, larva just fixing itself, flagellated half flat. D, fixed larva, flagellated cells beginning to invaginate. E, "Rhagon" stage. F, G, two stages in subdivision of Rhagon cavity by downgrowth of septa; *fl*, flagellated cells; *gr*, granular cells; *mes*, "mesenchyme"; *s*, septa dividing cavity of Rhagon.

After fixation the flagellated cells collapse to form a solid mass, which is speedily separated into smaller masses by ingrowths of the dermal cells, and these masses become hollowed out to form the spherical flagellated chambers.

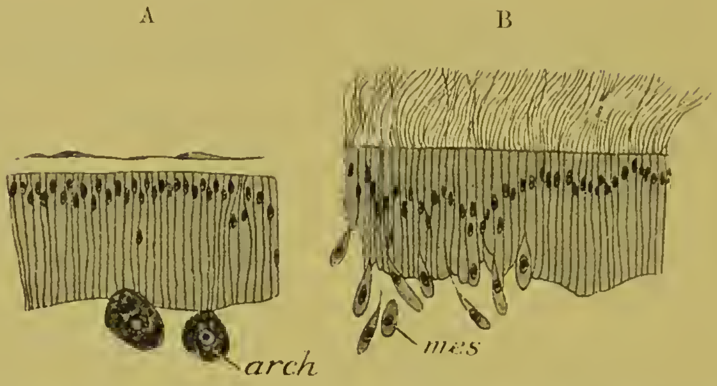

FIG. 23.—Two sections of the body-wall of the larva of *Plakina monolopha* in order to show the distinction between archaeocytes and mesenchyme. (After Maas.)

A, a piece of wall of embyro not yet hatched. B, a piece of wall of free larva; *arch*, archaeocytes; *mes*, mesenchyme.

In this series, *Grantia* forms, not the beginning, but takes the second place, and, viewing the series as a whole, we see a *progressive shortening of the larval life* joined to *an anticipation of adult characters.* We have, indeed, before us, typical examples of the commonest form of the modification of developmental history from its primitive form. This consists in the reflecting back of structures characteristic of one period of the life-cycle to successive earlier periods in ontogeny. It is called **heterochrony,** and its possible cause will be discussed in the summary. The merit of having called attention to it, and of having emphasised its importance, belongs to Lankester.

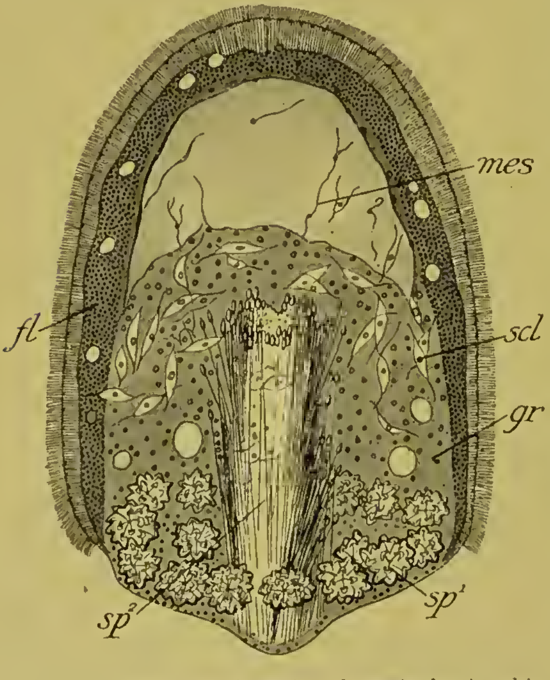

FIG. 24.—Longitudinal section through the Amphiblastula larva of *Esperia lorenzi.* (After Maas.)

fl, flagellated cells; *gr*, granular cells; *mes*, "mesenchyme"; *scl*, scleroblasts secreting curved spicules; sp^1, shovel-shaped spicules; sp^2, pin-head spicules.

The development of the most primitive sponges, the Asconidae, has been worked out by Minchin (1896), and his results are of great interest but a little difficult to reconcile with the series determined by Maas. In the genus *Clathrina*, the embryo is hatched as an oval ciliated blastula, with two cells at its posterior pole which are interpreted as the mother cells of the archaeocytes or primitive ova. From these cells numerous granular cells are budded off and fill the interior of the vesicle, but other cells formed by the modification of individual flagellated cells here and there, which lose their flagella, also migrate inwards. These latter cells, at fixation, are stated to burst forth and surround the ciliated cells.

In *Leucosolenia,* on the other hand, the posterior part of the interior of the blastula is filled with a mass of granular cells with small nuclei, and in front of these is a tube of flattened pigmented cells containing a lens-like body. This, according to Minchin, constitutes a rudimentary visual organ; it disappears at fixation. The cells of the posterior half of the blastula wall become granular *in situ* during the free life of the larva, and so an amphiblastula is produced. (Fig. 25.)

Further details of these interesting life-histories are urgently called for. We wish to know what corresponds to the archaeocyte in *Grantia.* If in this form the archaeocytes are only differentiated from the dermal cells after fixation, this must surely be a more primitive arrangement than what obtains in the Asconidae or in the Tetractinellida, where these primitive ova are differentiated during the segmentation of the egg. Minchin, indeed (1900), suggests that the granular cells, which are invaginated whilst the embryo of this sponge is in the tissues of the mother, are archaeocytes and are quite distinct from the cells forming the one end of the amphiblastula which he regards as transformed flagellated cells; but this view is negatived by Dendy's researches, the results of which have been described above.

The development of the well-known freshwater sponge *Spongilla,* which has been worked out in great detail by Evans (1899), presents several features of great interest. This sponge belongs to the group Demospongiae and forms a larva somewhat like that of *Esperia,* but the outer flagellate layer extends all round. One end of the larva is broader than the other and under this end is a cavity. The rest of the interior is filled with yolk-bearing "archaeocytes," whilst just under the skin is a layer of flattened cells with dense nuclei, like those described in the interior of the larva of *Grantia.* It metamorphoses in much the same way as *Esperia, i.e.* it fixes itself by the broad end; but Evans maintains that some of the "flagellate chambers" are formed at the expense of groups of archaeocytes and do not owe their origin to the flagellated epithelium which invested the surface of the larva.

Spongilla also reproduces itself by buds termed **gemmules.** The development of these in the allied genus *Ephydatia* has been worked out by Evans (1900). The gemmule first appears as a number of wandering cells in the jelly of the sponge, which are distinguished from their neighbours by possessing deposits of yolk in their cytoplasm. These cells gradually collect at a fixed point in the tissues, which is the centre for the formation of the gemmule. Here they become massed together so as to form a spherical lump. Other wandering cells with less yolk follow after them and form a layer surrounding them, in which the individual cells, as a consequence of mutual pressure, assume a columnar form. The investment is for a considerable time not quite complete, and an opening therefore exists. In this opening are found some specially large cells called **tropho-**

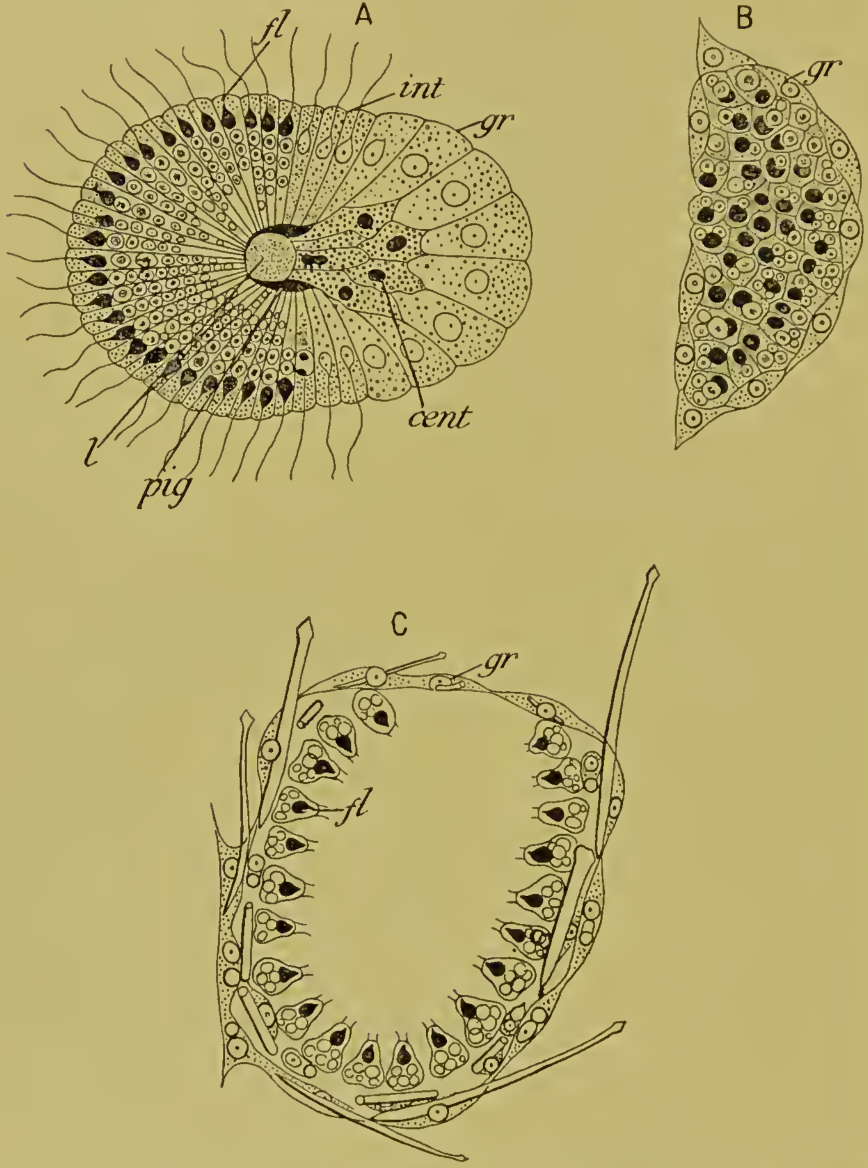

FIG. 25.—Longitudinal sections of Amphiblastula larva, just fixed larva, and young sponge of *Leucosolenia variabilis*. (After Minchin.)

A, free-swimming larva. B, just fixed stage. C, young sponge four days old; *cent*, central cells; *fl*, flagellated cells; *gr*, granular cells; *int*, intermediate cells; *l*, lens; *pig*, pigmented cells.

cytes, devoid of yolk, but with plentiful dark granules in their cytoplasm, which appear to act as carriers of nutriment to the enclosed yolk cells. The investing cells secrete a membrane on their inner surfaces. The columnar layer is then invaded by another class of wandering cells which come from the adjacent tissues of the sponge. These are clear cells carrying in their interior peculiar spicules called **amphidiscs**. The amphidisc resembles a pair of toothed wheels joined by an axle. Various stages in the development of amphidiscs can be seen in the maternal tissues. They first appear as little needles, similar in shape to the other spicules of the sponge. The ends of the needles thicken and eventually form wheel-like discs (Fig. 26 *amph*). When the amphidiscs have taken up their position amongst the cells of the investing layer, the cells which carried them degenerate and disappear.

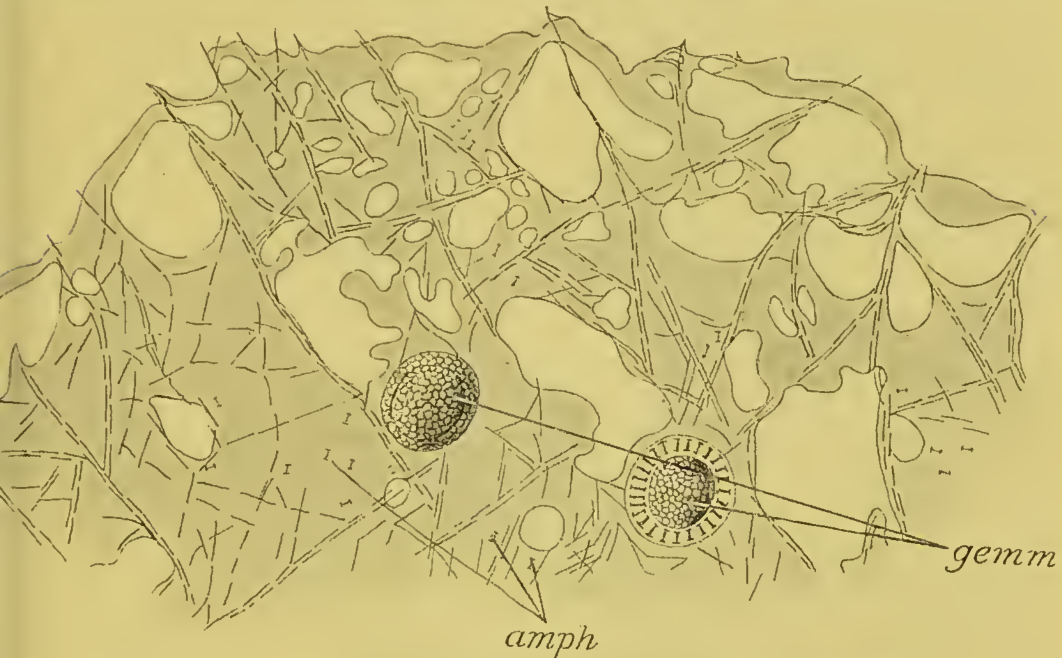

Fig. 26.—Section through a gemmule-bearing individual of *Ephydatia blembingia*. (After Evans.)

amph, Amphidisc; *gemm*, gemmule.

Before the investment of columnar cells is quite complete, the trophocytes withdraw from the yolk cells and pass back into the mother sponge. The inner ends of the columnar cells, after having secreted the membrane, likewise degenerate and form a sort of network of fibres between adjacent amphidiscs. The outer portions of these cells, however, become segregated off from their inner degenerating portions and pass back into the mother sponge. Before doing so, however, they secrete on their inner ends another membrane, which may be called the outer membrane of the gemmule, since it unites together the outer ends of the amphidiscs. Where the investing layer is finally completed no amphidises are found, and this forms a weak spot through which the inner mass, which is the real bud, eventually issues forth. The gemmules are set free on the decay of the parent in the autumn, fall to the bottom of the pool or stream and remain dormant till the spring. The inner mass then perforates the weak spot in the membranes, it streams forth in amoeboid fashion and forms a little mass of cells which develops into a sponge.

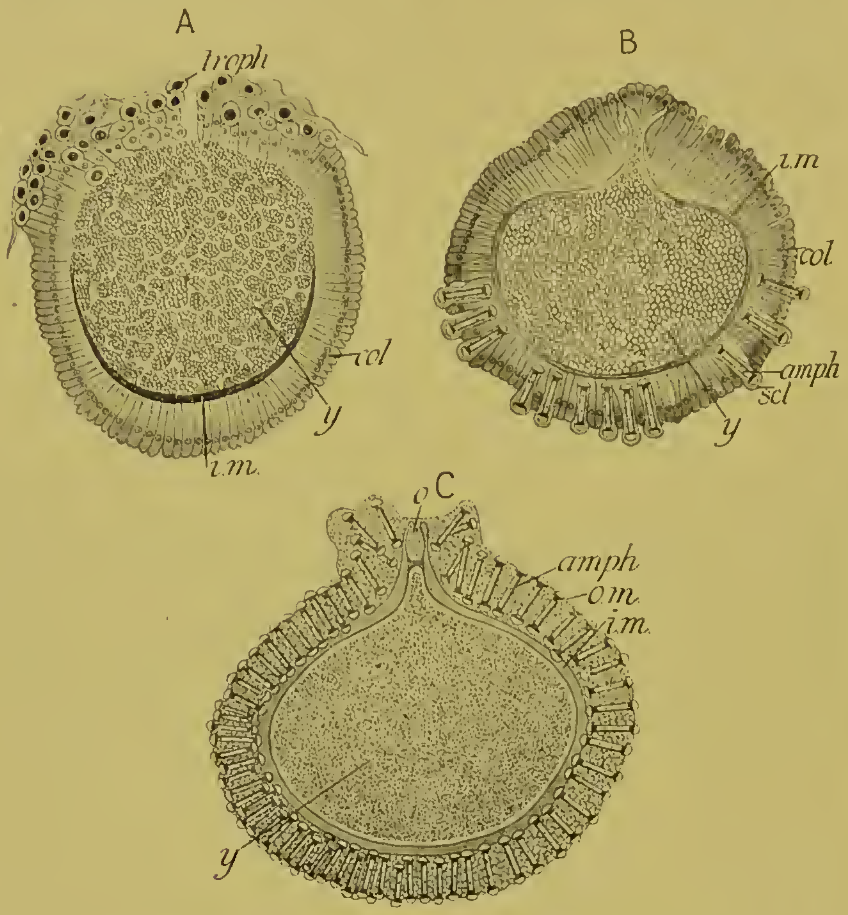

FIG. 27.—Three stages in the formation of the gemmules of *Ephydatia blembingia*. (After Evans.)

A, investment of columnar cells incomplete, trophocytes in contact with yolk cells, inner membrane being formed. B, investment of columnar cells complete; immigration of scleroblast with amphidises. C, ripe gemmule. Yolk cells form a solid mass; *amph*, amphidisc; *col*, columnar cells; *i.m*, inner membrane; *o*, aperture for escape of embryo; *o.m.*, outer membrane; *scl*, scleroblast; *troph*, trophocyte; *y*, yolk cells.

Marshall (1884) has shown that in *Spongilla lacustris* the sponges to which the gemmules give rise reproduce themselves by sexual cells and then perish, whilst the larvae which arise from the fertilized eggs grow into sponges which produce gemmules; thus there is in this sponge an **alternation of generations** similar to that with which we shall become familiar when we study the next group, Coelenterata.

Maas (1906) has reared the larvae of Calcareous Sponges in water artificially deprived of all carbonate of lime. The result was that no calcareous spicules were formed, and when the larva fixed the flagellated cells formed a solid mass and developed no lumen. Hence Maas concludes that the formation of spicules acts as a stimulus which determines the invagination of these cells to form a hollow cylinder. This may be true for *Grantia* and other Calcareous Sponges, but it is obviously untrue for *Oscarella* which has no spicules.

ANCESTRAL HISTORY

In the introductory chapter it was pointed out that there is strong evidence that larval forms are, broadly speaking, reminiscent of ancestral conditions of the stock or phylum. When we find in the ontogeny of all sponges the blastula form cropping up, and further find that, in those with the longest larval history this stage is *larval*, becoming embryonic only in cases of a short free life, we feel justified in assuming that it represents in a rough sort of way the common ancestor of all Porifera.

Such an ancestor—a hollow vesicle of flagellated cells—were it now living would be termed a colonial Protozoön. In *Volvox* we have an organism which, if it did not possess chlorophyll and live like a plant, would correspond fairly closely to our idea of what this ancestral sponge must have looked like. Now the great interest attaching to the blastula is that it appears as a *larva* in the life-histories of at least two other primitive groups of Metazoa, and that as a more or less modified embryo it can be detected in the development of *all* the Metazoan groups. Hence the case for regarding it as representing the ancestral stock of Metazoa is greatly strengthened.

But such a stock when it existed must have been of world-wide distribution, swarming in all the seas and waters of the globe. Such a world-wide stock would become adapted to different "stations" and just as at the present day we have bottom-feeding as well as mid-water fish, so we may imagine that bottom-feeding blastulae were developed. These, instead of devouring the floating and swimming organisms like the rest, turned their attention to the microscopic forms lying on the bottom. Under these circumstances only the cells on the lower half of the blastula would be effective feeders, and the more flattened this part became the more effective would be their work. The other cells would become merely protective and would tend to lose their flagella, and so the spherical blastula would be modified into a cap-like form. Fixation would be the next step, and so far as we can tell from a consideration of the life-histories of fixed animals belonging to other phyla, fixation is an adaptation to withstand and at the same time take advantage of currents. The larva, instead of creeping about seeking fresh food, holds on with its protective cells and lets the current waft fresh food into its reach.

So far our reasoning appears safe. But the porocytes baffle explanation; we cannot picture to ourselves a process by which cells converted themselves into drain-pipes, when we remember that every step in the process must have been functional and must have had a survival value. We can only imagine that the hollowing out of a cell is perhaps a shortened reminiscence of the process by which *gaps* in the attached rim, which must have existed to allow the ingress of water, became surrounded by protoplasm. The need for extending the surface of absorption, once fixation were accomplished, would account for the extension of the area of flagellated cells by their invagination, so that collectively they took on the form of a cylinder; but the formation of the osculum is utterly obscure.

In some few cases we can compare ancestral history as recorded by fossils, with ancestral history deduced from embryology; we can then see, as compared with the record deduced from fossils, what an abbreviated sketch is constituted by the embryological record. In the present case it is true we have no fossils to guide us, but the abbreviation of ancestral history, as reflected in larval history, must be intense. The later stages of the history of the race, the gradual complication of the chamber system, is mirrored in the post-larval development: the first fixed *Grantia* is at first an Ascon, and only gradually takes on the Sycon characters as it grows in size.

The history of all sponges just after fixation would be a most interesting field for research, and would throw much light on their mutual affinities.

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CHAPTER IV

COELENTERATA

Classification adopted—

- **Hydrozoa**
  - **Hydrida**
  - **Hydromedusae**
    - **Gymnoblastea**
    - **Calyptoblastea**
  - **Narcomedusae**
  - **Trachymedusae**
  - **Siphonophora**
- **Scyphozoa**
- **Actinozoa**
  - **Alcyonaria**
  - **Zoantharia**
    - **Edwardsiae**
    - **Hexactiniae**
    - **Cereanthidae**
    - **Zoanthidae**
- **Ctenophora**
  - **Cydippidea**
  - **Lobata**
  - **Cestidea**
  - **Platyctenea**
  - **Beroidea**

The Coelenterata are considered by many zoologists to be closely related to the parent group from which the other groups of Metazoa have sprung. In simplicity of general organisation they rival the Porifera, since the bodies of the adult Coelenterates, like those of Porifera, are composed of two layers of cells with an intervening jelly. Ever since in 1859, Huxley compared these two layers in a Coelenterate to the two primary layers of the Vertebrate embryo, they have been termed **ectoderm** and **endoderm**.

The most interesting thing about the relationship between the Porifera and the Coelenterata is that whilst the earliest stages of development in the most primitive representatives of each group are strikingly similar, and whilst in both cases a two-layered adult condition is reached, yet the steps by which this goal is attained differ so totally in the two cases that the two layers cannot be regarded as corresponding to one another in the two groups.

I. HYDROZOA

TUBULARIA

The type which we select for special descriptions is the common hydroid *Tubularia*, species of which are abundant in shallower water on British, Mediterranean, and American coasts. We base our account on the careful work of Brauer (1891) who has worked out the development of the Mediterranean species of *Tubularia mesembryanthemum.*

The British *Tubularia indivisa* is found attached to the bottoms of old boats. The medusa in most species of this genus remains permanently attached and the young pass through the earliest stages of their development within the bell of the mother. In this respect, as in the permanent attachment of the medusae, *Tubularia* is far from exhibiting primitive or typical conditions, but the free-swimming medusae of any particular species cannot always be obtained, and the eggs of these can often only be reared through the earlier stages of development. In many cases the hydroid stage is unknown, and a picture of the complete development of a typical hydroid which produces free-swimming medusae, can only be made out by piecing together the only fragments of the life-histories of many species which are known. It is for this reason that, in spite of its manifest disadvantages, we choose *Tubularia* as a type.

The egg and early segmentation stages are found by examining transverse sections of the **gonophores** and by means of whole mounts. The gonophores are the rudimentary medusae which remain attached to the colony throughout life. On the manubria of these the eggs and sperm are produced. The eggs are dehisced into the bell, in which they undergo practically the whole of their development, so that the bell is no longer a *locomotor organ* but a *nursery.* The eggs are amoeboid and appear to segment fairly regularly, but they are deformed by mutual pressure in the confined space in which they find themselves. Only two or three are dehisced at one time.

The segmentation of the egg is somewhat irregular and leads, after about sixteen segments have been formed, to the formation of a hollow vesicle or **blastula.** It is, however, a remarkable circumstance that what appears to be an abnormal form of segmentation frequently occurs and leads also to the formation of a regular blastula. In this latter form of development the nucleus divides repeatedly before any division of the protoplasm occurs, and then subsequently the multinucleate mass is cut into cells. When we find that these two methods of development sometimes characterize different genera of the same class of the animal kingdom, we are apt to think of them as very different, but their occurrence side by side in the same species shows that the physiological difference separating them must be very slight.

The blastula stage is succeeded by a solid **morula** stage. This word, which literally means "mulberry," is used to characterize a condition where the cells which constitute the embryo form a compact spherical mass. The morula stage is reached by the proliferation of cells from the walls of the blastula in sufficient number to fill up the interior. Whether these cells are budded from all parts of the blastula wall or only from a certain area of it, has not been made out. In the case of the eggs of the free-swimming medusae, however, it is beyond all question that these cells are budded only from one end of the blastula, and none of Brauer's figures are inconsistent with the assumption that this is the case with the blastulae of *Tubularia* also. In any case a solid morula stage is soon reached in which the whole interior of the blastula becomes clogged up with a mass of cells.

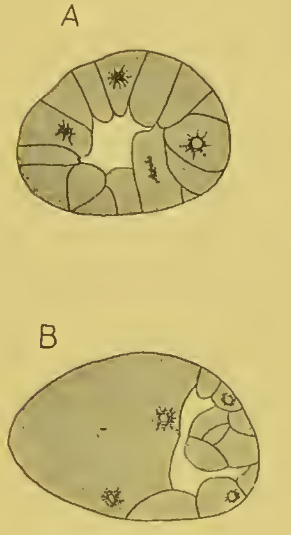

Fig. 28.—Two methods of formation of the blastula in *Tubularia mesembryanthemum*. (After Brauer.)

A, egg segmenting in normal method. B, egg segmenting in abnormal method.

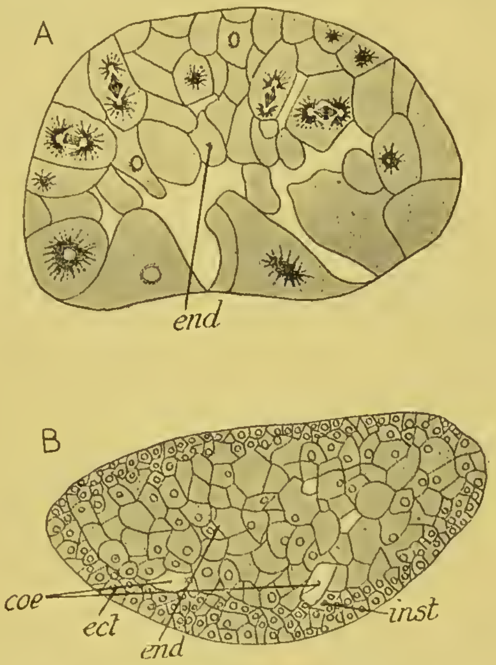

Fig. 29.—Formation of endoderm in *Tubularia mesembryanthemum*. (After Brauer.)

A, budding of endoderm cells from blastula wall. B, morula stage. *coe*, spaces which will ultimately form the coelenteron; *ect*, ectoderm; *end*, endoderm; *inst*, interstitial cells.

This mass of cells constitutes the rudiment of the **endoderm** of the adult, whilst the original blastula wall forms the **ectoderm.** In the solid mass of endoderm spaces begin to appear owing to the absorption of some of the central cells. These spaces (*coe*, Fig. 29) eventually coalesce so as to form one cavity which is the gastral cavity or **coelenteron** of the adult. The embryo has now the form of a circular disc, and from its edges a series of blunt protuberances grow out. These are the rudiments of the **aboral tentacles** of the adult: they are bent towards the future aboral end of the body. At the opposite or oral end is a spot where ectoderm and endoderm thin out, and there eventually the **mouth** will be formed by a perforation of both layers. The disc-shaped embryo grows rapidly in the direction of its

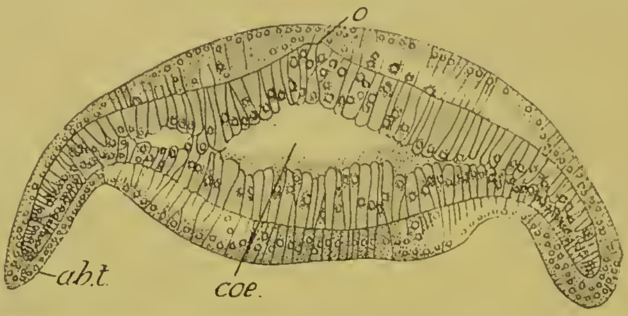

FIG. 30.—Section of embryo of *Tubularia mesembryanthemum* showing the formation of the aboral tentacles. (After Brauer.)

ab.t, aboral tentacle; *coe*, coelenteron; *o*, spot where the mouth will be formed.

principal axis and becomes cylindrical, and finally develops round its oral end a series of small protuberances which are the rudiments of the **oral tentacles** of the adult. By this time the mouth has been formed and the aboral tentacles have become long and slender (Fig. 31).

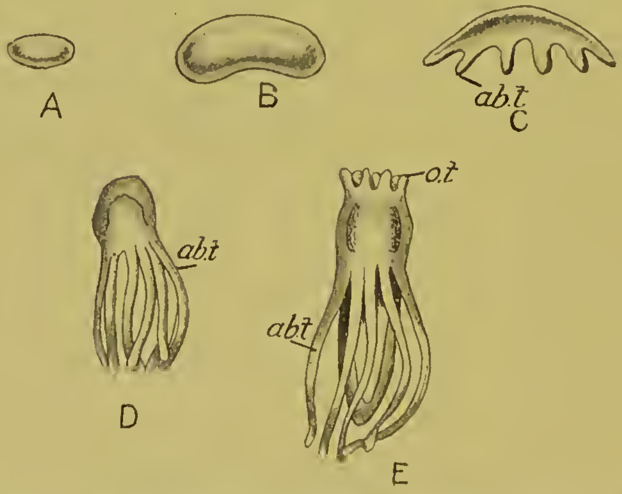

FIG. 31.—Five views of external features of different stages in the development of the embryo of *Tubularia indivisa*. (After Allman.)

A, oval embryo. B, aboral concavity appears. C, rudiments of aboral tentacles. D, tentacles long; embryo becomes cylindrical. E, oral tentacles formed. *ab.t*, aboral tentacles; *o.t*, oral tentacles.

The embryo is now called an **Actinula**, and is ready to leave the bell of the gonophore. It escapes from its nursery, and creeps about on the bottom of the sea with its mouth turned downwards, but finally it attaches itself by the aboral end. It then rapidly grows in height, and from its sides daughter persons are budded, so that it forms an upright shoot in the adult colony. From its base arise creeping **stolons** which give rise at intervals to other upright shoots. Its lower part secretes a horny shell, the **perisarc.**

The adult colony, however, is still unripe sexually; full sexual maturity is only reached by the gonophores produced subsequently, and thus we have an **alternation of asexual and sexual generations.**

The development of these gonophores in the case of *Tubularia* (as well as in the case of many other genera) has been worked out by Götte (1907); we follow his account in what follows. From just above the region of the aboral tentacles, finger-like stolons grow out, and on these, lateral protrusions arise, which are the **medusa-buds.** At the tip of such a bud, the ectoderm thickens to form a mass of cells, and in this mass a cavity develops, the future **umbrella-cavity.**

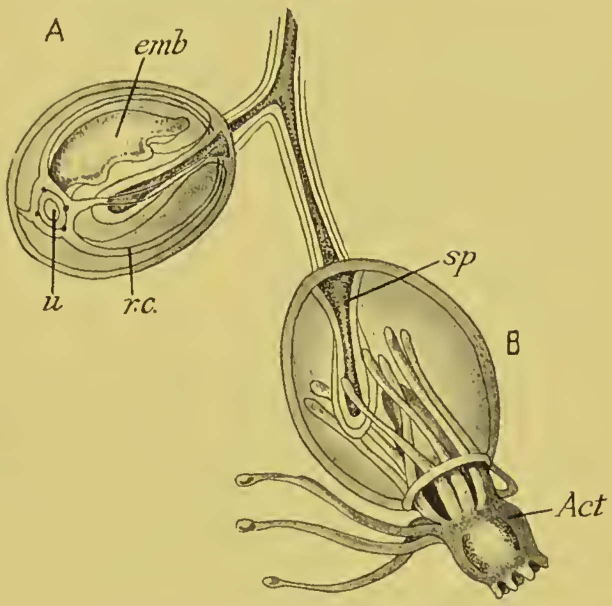

FIG. 32.—Two gonophores of *Tubularia indivisa* with developing embryos inside. (After Allman.)

A, gonophore with discoid embryo ; opening of umbrella-cavity just formed ; radial canals clear. B, gonophore with actinula larva just escaping ; radial canals have disappeared. *Act*, actinula ; *emb*, embryo ; *r.c*, radial canal ; *sp*, manubrium or spadix ; *u*, opening of umbrella-cavity.

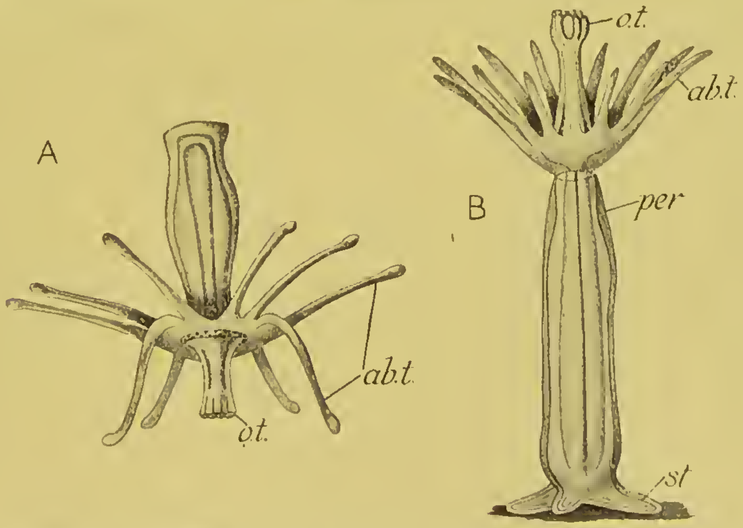

FIG. 33.—Stages in development of Actinula larva of *Tubularia indivisa*. (After Allman.)

A, creeping larva. B, first fixed form. *ab.t*, aboral tentacles ; *o.t*, oral tentacles ; *per*, perisarc ; *st*, stolon.

The mass is termed by German authors "**Glockenkern**," which we may translate "**bell-rudiment**"; it is crescentic in section with its concavity directed downwards. Into this concavity fits a protrusion of the endoderm of the medusa-bud; this is the "**spadix**," the rudiment of the future manubrium, so far as its endodermal portion is concerned. From the base of the spadix four hollow protrusions of endoderm grow out as canals, and insinuate themselves between the ectoderm of the bud and the outer ectoderm of the bell-rudiment. The spots where these canals bud out are the interspaces between four vertical, solid ridges of endoderm called the **taeniolae,** which project into the gastric cavity of the spadix.

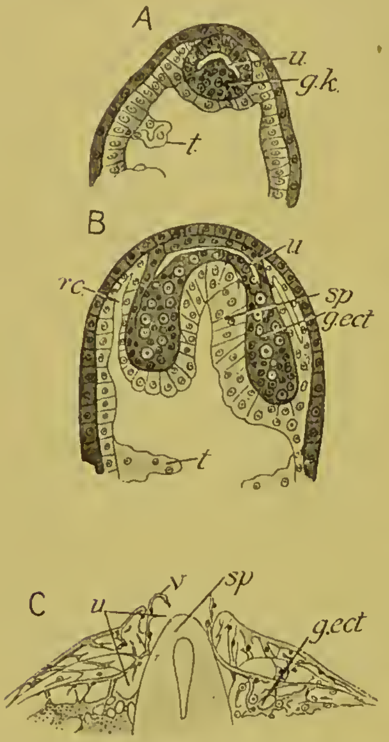

FIG. 34.—Three longitudinal sections through developing medusa-bud of *Tubularia mesembryanthemum*. (After Götte.)

In A, the youngest, the bell-rudiment is just formed. In B, the spadix and radial canals are differentiated. In C, the umbrella-cavity is open to the exterior. *gk*, bell-rudiment (Glockenkern); *g.ect*, genital ectoderm; *r.c*, radial canal; *sp*, spadix; *t*, taeniola; *u*, umbrella-cavity; *v*, velum.

The canals are termed the **radial canals,** and they eventually push out short protrusions of the ectoderm which are the rudiments of the **medusa tentacles.** The radial canals flatten out and become fringed with flat extensions of endoderm. These extensions meet one another in the centre of the interradii, and so constitute continuous sheets of endoderm covering the interradii and forming the so-called **endoderm lamella** (Fig. 35, *en.l*).

The apex of the medusa-bud consists only of the ectoderm of the bud and the outer ectoderm of the bell-rudiment closely pressed together. Absorption of these adpressed layers now takes place, and an opening is thus formed which places the cavity of the bell in connection with the exterior, and through this aperture the manubrium often protrudes. The thin ectoderm round the edge of the aperture forms the **velum.** In *Tubularia* the hollow radial canals are transitory structures, and their lumina soon become crossed by cords of cells.

The **genital cells** are found in the ectoderm covering the lower part of the spadix. According to Brauer (1891) they originate as interstitial cells of the ectoderm in the early stages of development of the medusa-bud; they then migrate through the jelly into the endoderm, and finally into the ectoderm covering the spadix (Fig. 36).

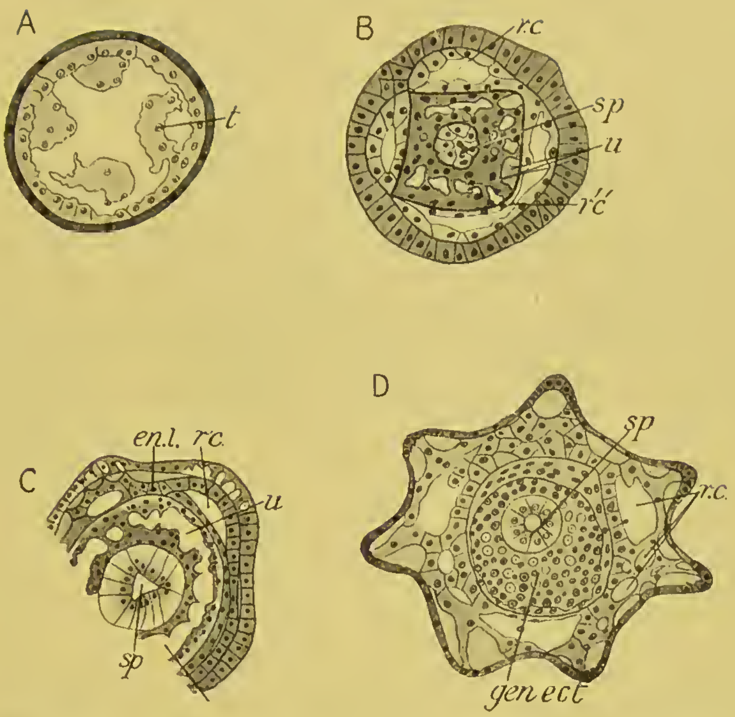

FIG. 35.—Four transverse sections through the developing gonophore of *Tubularia mesembryanthemum*. (After Götte.)

A, section through base of gonophore showing the four taeniolae; *t*, taeniola. B, section through upper part of older gonophore; *r.c*, radial canal; *r'c'*, spot where two radial canals join; *sp*, spadix; *u*, spaces, portions of the irregular umbrella-cavity. C, section through still older gonophore; letters as before; *en.l*, so-called endoderm lamella. D, section through almost mature gonophore. *r.c*, remnants of cavity of radial canals; *gen.ect*, generative ectoderm clothing the spadix.

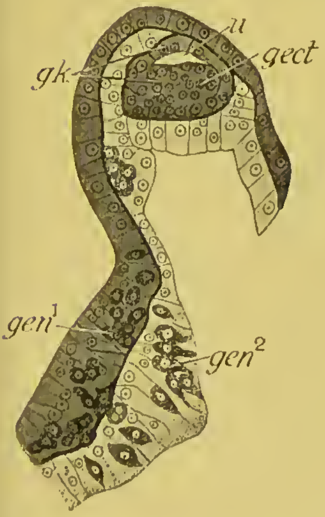

FIG. 36.—Longitudinal section through very young gonophore-bud of *Tubularia mesembryanthemum* to show the origin of the genital cells. (After Brauer.)

Letters as in Fig. 34. In addition: *gen*[1], genital cells originating in ectoderm; *gen*[2], genital cells which have penetrated into endoderm.

FREE MEDUSAE

When we contrast with this development the life-history of the free medusae so far as it is known, we find many marked differences. Our principal source of information on this subject is Metschnikoff (1886), who captured the free medusae of *Tiara*, *Rathkea*, *Oceania*, *Clytia*, etc., and kept them in aquaria till they had deposited their eggs. He was then able to rear the embryos through the larval stages, until they produced young hydroid colonies.

In nearly every case he found that the egg underwent a very regular segmentation which led to the formation of a **blastula**; this was at first spherical but soon became oval and ciliated, with an anterior broad and a posterior more pointed end, and which swam freely about in the water. At the pointed end, *and at this end alone*, cells migrated inwards and formed a mass which rapidly increased in extent, owing, not only to the successive immigration of new cells, but also to the division of the immigrated cells *in situ*; and so the blastula was converted into what is termed a **planula**.

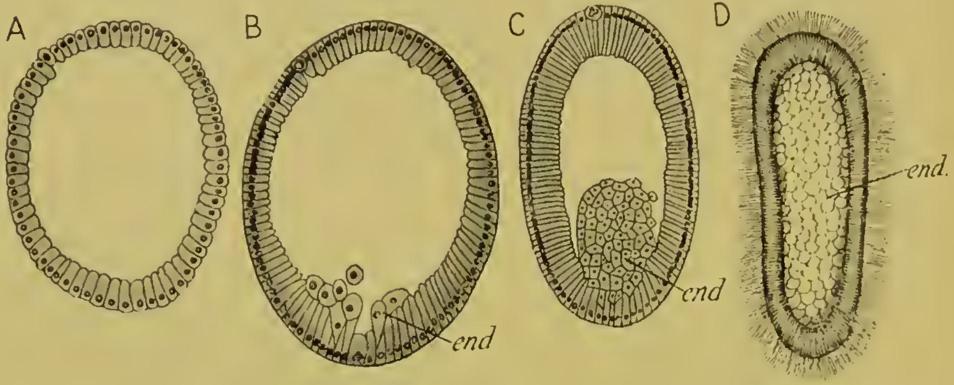

FIG. 37.—Four stages in the development of the planula of *Clytia*. (After Metschnikoff.)

A, blastula stage. B, formation of endoderm by immigration of cells of blastula wall at one pole. C, endoderm, a solid mass, half-filling the cavity of the blastula. D, free-swimming planula larva. *end*, endoderm.

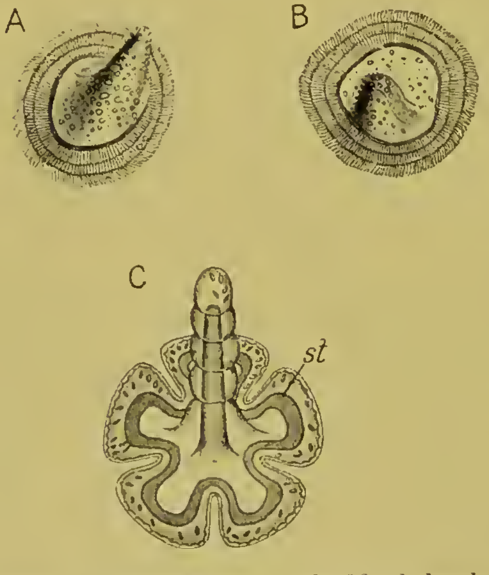

FIG. 38.—Three stages in growth of fixed planula of *Clytia*. (After Metschnikoff.)

A, at moment of fixation. B, a short time after. C, a day after. *st*, divisions of broad attached end which are the rudiments of stolons.

In the vast majority of Hydrozoa, and also in many Actinozoa, the organism enters on its free life in the "planula" stage. A planula is an oval larva covered with a layer of ciliated cells containing a solid mass of cells inside. Development within the bell of the parent medusa till the adult form is attained, such as occurs in *Tubularia*, is exceptional. Hence the planula is termed the typical larva of the Coelenterata. This planula, after a short free life, attaches itself to the bottom by the broad end, which flattens out. Then, and then only, absorption of the central cells takes place, and a gastric cavity makes its appearance. The broad end becomes divided by indentations into lobes, each of which constitutes one of the creeping stolons. The narrow end grows up and develops into the first polyp, the mouth and tentacles being formed as described in *Tubularia*. Metschnikoff's results have been confirmed in the most gratifying manner by Rittenhouse (1910), who studied the development of the eggs of the medusa *Stomotoca apicata*. The sole point in which he is inclined to differ from Metschnikoff is, that he regards the endoderm as arising by the budding of cells from the cells constituting the blastula wall, rather than from the migration of cells forming part of that wall into the interior. Thus we see that processes, which in the case of *Tubularia* are completed before the larva leaves the bell of the mother, do not occur in the case of the free medusae till long after the larva is fixed.

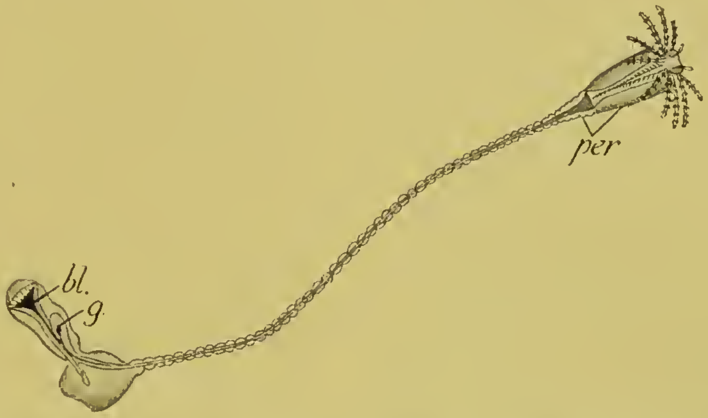

FIG. 39.—A young colony of *Clytia* reared from a planula in the aquarium. (After Metschnikoff.)

bl, blastostyle; *g*, rudiment of medusa; *per*, perisarc.

A thorough study of the development of the medusae and gonophores has been made by Götte (1907). In the series of progressive modifications of development which can be constructed from the development of the forms which he describes, *Tubularia* and *Pennaria* (in which the medusae occasionally become free) take the second place. The most primitive type of development is found in forms like *Podocoryne*, in which the medusa regularly becomes a free-swimming organism, and swims about for a long time, and eats and grows before it develops genital cells.

In *Podocoryne*, after the medusa-bud has attained the stage just described for *Tubularia*, after the radial canals have been formed, they give rise to lateral outgrowths which meet those of adjacent radial canals, and in this way a circular canal is formed; then the freely-projecting ends of the radial canals give rise to free tentacles. By the formation of flat solid extensions from the lateral walls of the radial canals, which meet each other, a continuous sheet of endoderm is formed which spreads over the whole extent of the bell. This is called the **endoderm lamella**. The manubrium attains a mouth, and the whole medusa becomes free by the absorption of the stalk of the bud. Generative cells are only matured after a considerable period of free-swimming life.

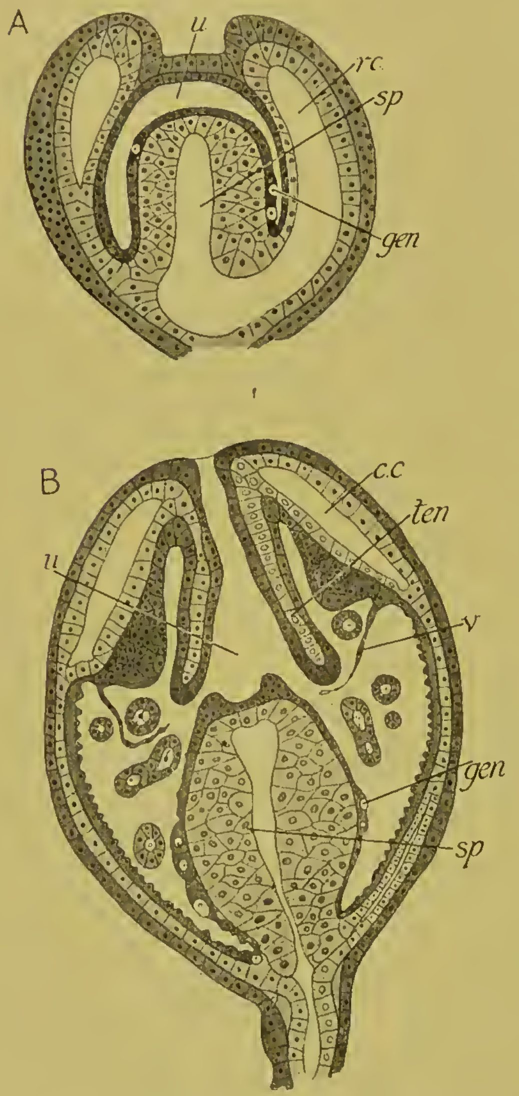

Fig. 40.—Two longitudinal sections through the developing medusa-bud of *Podocoryne carnea*. (After Götte.)

A, before umbrella-cavity is open to the exterior. B, after umbrella-cavity is open to the exterior. *c.c*, circular canal; *gen*, developing genital cells; *r.c*, radial canal; *sp*, (in A) cavity of the spadix, (in B) wall of the spadix; *ten*, tentacle; *u*, umbrella-cavity.

On the other hand, according to Götte, in *Clava* the bell-rudiment is formed but the umbrella-cavity never opens to the exterior, nor are there any radial canals formed, and the whole bell-rudiment is absorbed before the germ cells are shed. In such forms as *Clava* the generative cells of at least one sex—in the case of *Clava* of both sexes—can be detected in the stalk of the person (**blastostyle**) which bears the gonophores. Their development in this genus has been described by Harm (1903). They appear to arise from amongst the interstitial cells of the ectoderm, but, as is also the case with the genital cells of *Tubularia*, they migrate early into the endoderm, where they grow. They reach the spadix when fully ripe, and burst through the ectoderm there (Fig. 42).

In *Cordylophora* the bell-rudiment remains a solid mass of cells, part of which is converted into generative cells.

In *Sertularia* the gonophore is a broad-based lateral swelling on the blastostyle, the bell-rudiment separates from the ectoderm and splits imperfectly, the vestigial umbrella-cavity being crossed by cell trabeculae; and finally, in *Halecium* no bell-rudiment at all is formed.

The last two forms belong to that division of Hydromedusae termed Calyptoblastea, which possess **hydrothecae**, and in which the mouthless person, or **blastostyle**, which bears the gonophores, is enclosed in a special case, the **gonangium**. This gonangium is secreted by a special outer layer of ectoderm, the **mantle layer**, which breaks away from the inner ectoderm covering the medusa-bud. In *Halecium* a set of endoderm tubes, like the radial canals, grow out from the blastostyle and ramify in the mantle.

Götte interprets the series of forms which he describes, as steps in the building up of the medusa out of what was originally nothing but a lateral swelling on a hydroid **person** (as the member of a Coelenterate colony is termed). This swelling is caused by the genital cells, and is therefore similar to the swelling produced by the ovary or testis of *Hydra*. The majority of zoologists, however, read the series in the opposite direction and, as it seems to us, with infinitely more justice. They regard the "gonophores" as degenerate forms of medusae, which once were perfectly developed and became free, but have ceased to be detached, and so the structures which a free medusa uses for swimming have become functionless in them. How else can the umbrella-cavity of *Clava*, which never opens and becomes completely resorbed, be interpreted?

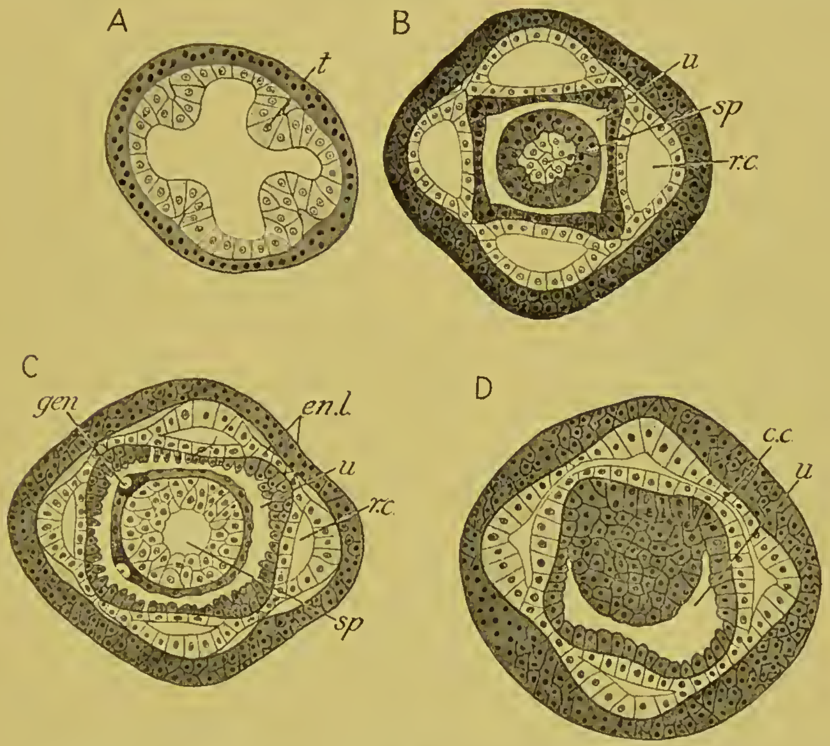

Fig. 41.—Four transverse sections through the developing medusa of *Podocoryne carnea* to show formation of *circular* canal and endoderm lamella. (After Götte.)

A, through base of young gonophore showing four taeniolae. B, through older bud showing four separate radial canals. C, through base of older bud showing the fringes growing out from the radial canals which form the endoderm lamella. D, through upper part of older bud showing circular canal. In this figure the roof of the umbrella-cavity is grazed. Letters as in previous figure; *en.l*, endoderm lamella; *t*, taeniola.

At the same time Götte's results have thrown light on how a medusa was developed out of a hydroid form. It used to be held that a medusa was essentially a hydroid shortened in the direction of the mouth-foot axis. This shortening, it was thought, had caused the oral and aboral walls of the peripheral portions of the stomach of the hydroid to adhere to one another, and so to form a solid plate of endoderm, the so-called **endoderm lamella**; thus leaving the lumen only in the centre, in the extreme outer edge (the circular canal), and in four radiating lines (the radial canals).

But if we follow Götte we must imagine a simpler process of evolution. The bell of a medusa is, according to him, merely a web connecting the basal parts of the tentacles of a hydroid, and a medusa is related to a hydroid as a duck's foot is related to a hen's foot.

We may suppose, then, that originally the hydroid persons were separated from the mother, and crept about, as still happens in the case of the buds of *Hydra*, and that these persons eventually developed genital organs; but that a differentiation in these buds took place, so that some never separated, but remained permanently immature and asexual, whilst those that did separate developed a swimming web. In this way the **alternation of generations** so characteristic of Hydrozoa was developed.

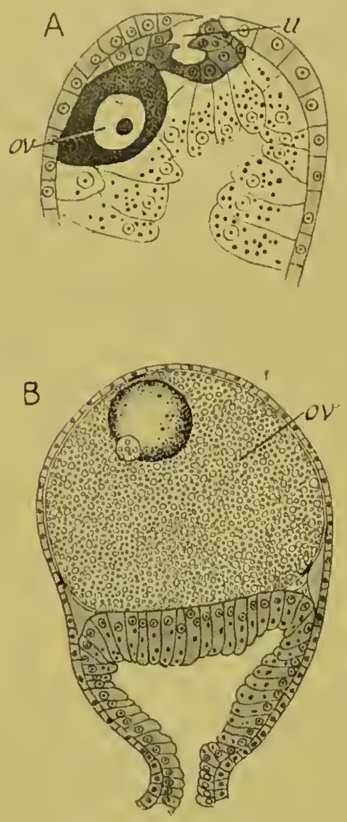

FIG. 42.—Two longitudinal sections through the developing gonophore of *Clava squamata*. (After Harm.)

A, young gonophore with rudimentary umbrella-cavity and unripe ovum embedded in endoderm. B, fully developed gonophore with ripe ovum. *ov*, ovum ; *u*, umbrella-cavity.

SIPHONOPHORA

The Siphonophora are floating or swimming Hydromedusae. The most ingenious and plausible hypothesis as to their origin is that put forward by Korschelt and Heider (1890), who regard as most primitive those forms like *Physalia* and *Velella*, which float only, and are without those engines of propulsion known as **nectocalyces**. Korschelt and Heider (1890) suppose such forms to have been derived from larvae of ordinary Hydromedusae, which have fixed themselves to the surface film of the water.

That this is a possible and even probable contingency will be self-evident to any one who has watched young starfish walking upside down on the surface film, like flies on the ceiling of a room, or who has seen some members of a swarm of Ascidian tadpoles thus fix themselves to the film. The surface film, although able to sustain the weight of a larva, would soon bend under the growing weight of the hydroid colony which developed from it, and this would lead to a cupping of the base. If we suppose this base to secrete mucus and to entangle bubbles of air, the elements of a float would thus be presented.

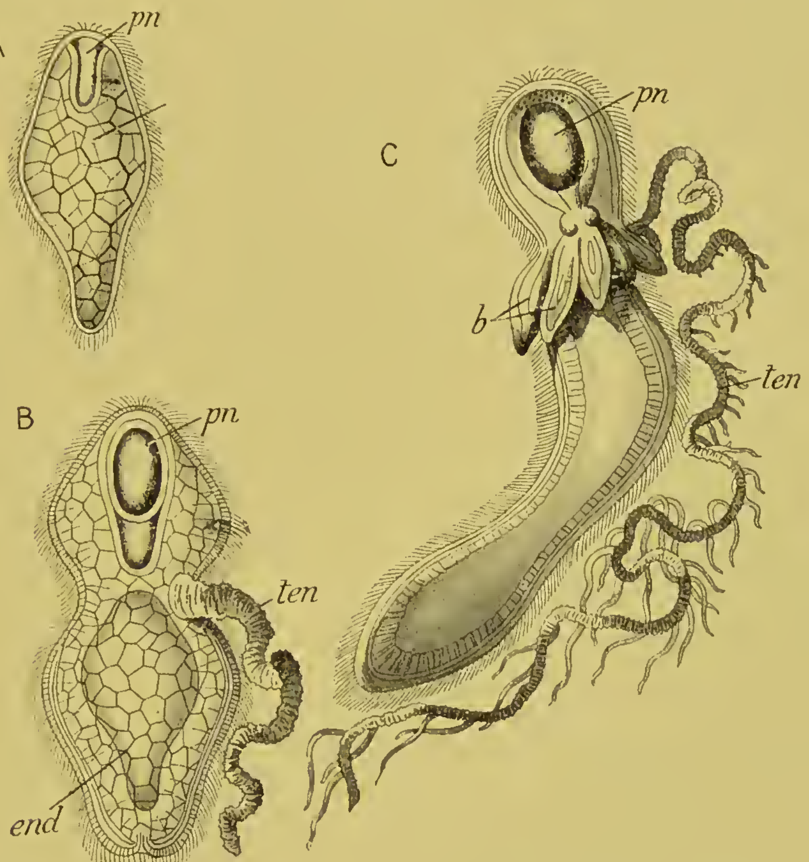

FIG. 43.—Three stages in the development of a Siphonophore (*Cystalia monogastrica*). (After Haeckel.)

A, planula with float, an open invagination of the aboral ectoderm. B, Older larva, a single long tentacle formed. C, still older larva in which the definitive endoderm is formed, and in which buds of other persons have been formed. *b*, buds ; *end*, endoderm ; *pn*, float ; *ten*, tentacle.

The earlier stages in the development of these Siphonophora have not been made out, but the planula larva is well known. The peculiarity of this larva lies in the fact that the large vacuolated internal cells which occupy its interior, are not directly converted into the endoderm of the adult, but that they bud off smaller cells on their outer sides, which form the definitive endoderm which persists throughout life.

As in other planulae, the narrow end lengthens and becomes converted into the body of the first hydroid polyp, at the apex of which the mouth is formed; but the broad end develops an ectodermic invagination, the rudiment of the **float**, at the spot where the attached base would naturally be looked for if we were dealing with planulae of Hydromedusae. A single tentacle sprouts from the base of the polyp, and above this, *i.e.* nearer the float, is a **growing zone**, from which other polyps arise.

In many Siphonophora certain of the medusoid buds lose their genital cells, and even the manubrium, and become organs of locomotion merely. These organs arise in the part of the growing zone nearest the float, morphologically the most basal part. In one group the adult relies on these modified medusae (**nectocalyces**) alone for swimming, the float having disappeared; and in such cases, which we regard as the most modified of all, the endoderm cells in the base of the planula secrete oil drops, and the first definite organ to be formed in this region is a huge nectocalyx.

To this theory of Korschelt and Heider there are opposed two other theories, viz. the medusome theory of Haeckel (1888) and the theory of Chun (1887).

According to Haeckel, the whole Siphonophore colony is merely a medusa which budded, as some few medusae are known to do. Every person is supposed to be a modified medusa; the bells of the medusae are supposed to be represented by the translucent leaf-like bracts, termed **hydrophyllia**, which many species possess; and the hydroid-like persons are their "manubria," which are supposed to have migrated out from them through a slit in the bell. The violent dislocations required by this theory belong to the period of imaginative morphology.

Chun agrees with Korschelt and Heider in regarding the Siphonophore as a Hydromedusan colony, containing both hydroid and medusoid persons; but he regards the float as a modified medusa, in which air has replaced water. It is, however, very difficult, if not impossible, to picture a series of ancestors in which one of the medusa bells gradually replaced its contained water by air. In other words, Chun's hypothesis transgresses the law of **functional continuity**, which should be exemplified in any supposed phylogenetic change.

NARCOMEDUSAE AND TRACHYMEDUSAE

The Narcomedusae and Trachymedusae are usually stated to be Hydromedusae, in which the egg develops directly into a medusa without an intervening hydroid stage. A more correct statement of the case would seem to be that the egg develops into a modified hydroid person, which does not bud, but which, by the formation of a web, becomes directly transformed into a medusa. The fact that both these groups are pelagic in their habit has rendered the formation of a fixed budding colony of hydroids impossible. Therefore the development is hurried on, and the first person develops into a medusoid, passing, in some cases at least, through a hydroid stage in the course of its development.

In the development of Geryonidae, a family of the Trachymedusae, it is usually stated that a spherical blastula is at first formed, and then that the inner vesicular portion of each cell of the blastula becomes detached from the outer end, and that these inner portions unite to form an endodermic vesicle. In this way, it is said, the two-layered condition is reached.

This view is founded exclusively on views of living segmenting eggs which, owing to their spherical character, could not be orientated; and on the assumption that this mode of the formation of endoderm and ectoderm actually occurs, it has been regarded as typical **delamination**, and as representing the primitive way in which a two-layered condition was arrived at.

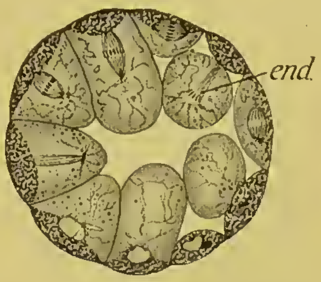

FIG. 44.—Embryo of a Geryonid (*Carmarina fungiformis*) in which endoderm cells are being budded off. (After Metschnikoff.)

end, endoderm.

Strong objections may be urged against this view. The development of the Trachymedusae and Narcomedusae is greatly modified as compared with that of the more normal Hydromedusae, on account of their mode of life; and further, when we consider how easily mistakes can be made as to the nature of a process, unless carefully orientated embryos are examined and cut into sections, we must regard it as very questionable whether the kind of delamination described in the Geryonidae does actually take place. It is possible that in this family we have to do with a proliferation of the cells forming the blastula wall, at one side of the blastula, but that the area of proliferation is of considerably greater extent, relatively to the whole surface of the blastula, than it is in the case of the blastulae of ordinary Hydromedusae. If this pole were turned towards the observer, he would receive the impression that he was looking at a sphere, from the whole of whose circumference cells were being budded inwards.

II. SCYPHOZOA

AURELIA

If we now turn our attention to the great group of the Scyphozoa, we find that the development of the genus *Aurelia* has been fully worked out, the latest accounts being given by Hein (1900) and Friedemann (1902). These workers used a mixture of 100 parts concentrated solution of corrosive sublimate, with 2 % acetic acid, to preserve the larvae of *Aurelia*.

This common jelly-fish swarms on both sides of the Atlantic. As in all Scyphozoa the genital cells are produced from the endoderm of the stomach, and are discharged into its cavity,— where they are fertilized by spermatozoa of other individuals taken in with sea-water. The fertilized eggs escape from the mouth but are retained for a considerable period in pockets of the inner surface of the oral arms. These pockets can be recognized in the surface view as opaque spots. If they are slit open by needles under sea water the embryos can be extracted.

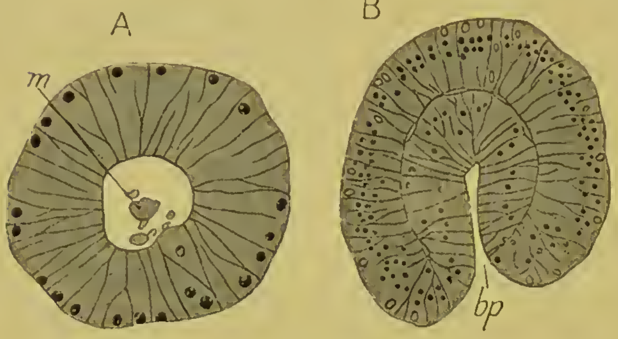

FIG. 45.—Early stages in the development of *Aurelia aurita.* (After Hein.)

A, blastula stage. B, gastrula stage. *bp*, blastopore; *m*, cells budded from the blastula wall which migrate into the interior and disintegrate.

The embryos can be preserved in corrosive sublimate and acetic acid, or in osmium acid, and mounted whole; or else embedded in celloidin, orientated, and cut into sections parallel to their longitudinal axis. When such sections are examined it is found that the egg segments with great regularity, and that a spherical hollow blastula is formed. The cells forming the outer wall of this blastula bud off other cells which migrate into the interior, and it looks as if we were about to witness the formation of a solid planula; but the cells which thus migrate inwards break up into granules and are absorbed, thus serving as food for the rest. Then, at one end, the cells forming the wall of the blastula are *invaginated*, and in this way the single-layered blastula is converted into a hollow, double-layered structure termed a **gastrula**. The opening of the invagination is termed the **blastopore**. The conversion of a blastula into a gastrula is called the process of **gastrulation**.

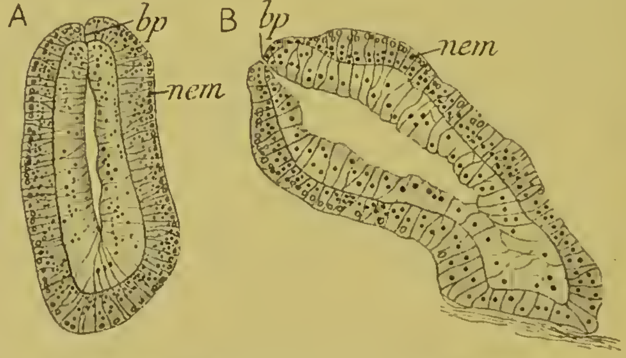

FIG. 46.—The fixation of the free-swimming larva of *Aurelia aurita.* (After Hein.)

A, free-swimming planula. B, stage just after fixation. *bp*, blastopore reduced to a mere slit. *nem*, nematocysts.

The blastopore never closes and eventually forms the **mouth,** although it becomes contracted to the finest capillary dimensions. The yolk granules in the cells become absorbed, and the spherical gastrula becomes converted into an oval one, with a broad basal end and a more pointed end where the mouth opens. Thus by a single process a stage is reached which, in the Hydromedusae, is attained first by a process of the immigration of cells, then by the absorption of the more central cells, and lastly by the formation of an aperture to the exterior.

The embryo now emerges from the maternal pockets and swims freely about by means of its cilia. The outer cells commence to show traces of the formation of nematocysts, whilst the inner cells develop large vacuoles, as in *Hydra*. It appears that, as in the blastula stage so also in the gastrula stage, cells migrate from the wall of the stomach into its cavity and are digested.

After swimming about for four or five days the larvæ attach themselves by their broad ends, the ectoderm cells of which secrete an adhesive secretion. The attached larva becomes gradually somewhat flattened, and passes from a cylindrical to a cup shape. The endoderm cells in the neighbourhood of the mouth multiply rapidly to form a slight elevation or **oral cone**. The ectoderm cells in this region do not multiply but become stretched so as to form a thin flattened layer. The almost obliterated blastopore becomes now widened so as to form the permanent mouth; it becomes indeed quite a gaping opening.

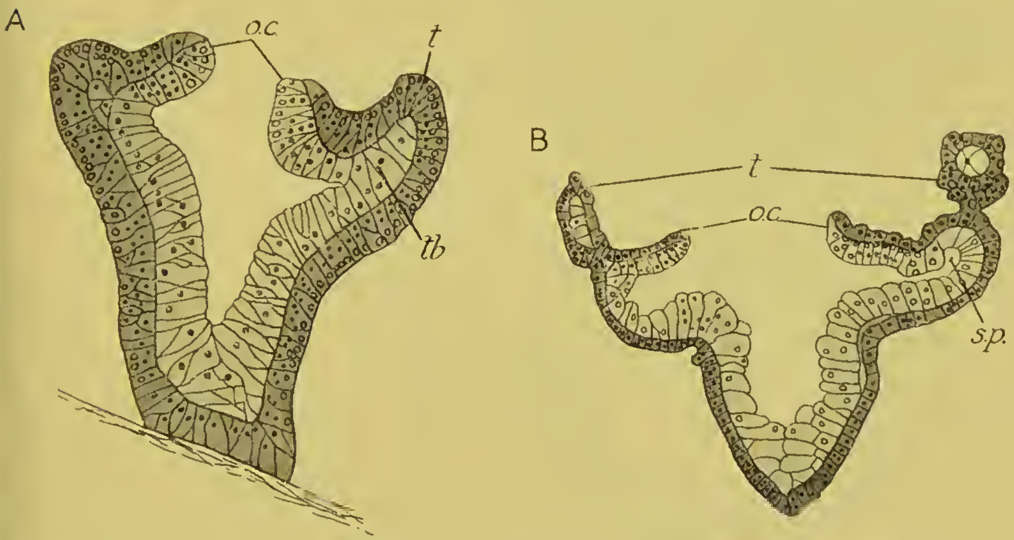

FIG. 47.—Two longitudinal sections through two Hydra-tubae of different ages.

A (after Hein), through a specimen with four tentacles. The section goes through the origin of a tentacle. B (after Friedemann), through a specimen with eight tentacles, showing the stomach pocket intervening between two taeniolae. o.c, oral cone; s.p, stomach pocket; t, tentacle; t.b, tentacle bases of vacuolated endoderm.

Immediately after this, four primary tentacles arise as warts surrounding the mouth. The interior of each is occupied by a solid cord of endoderm, and the ectoderm covering it becomes crowded with nematocysts. Alternating with these tentacles there arise four **taeniolae** or ridges of the endoderm projecting into the stomach cavity. Each of these is produced by an inwardly-directed fold of the endoderm, between the limbs of which is jelly. These taeniolae are also termed **septa** (Fig. 48).

Then a circular depression appears in the ectoderm on the upper part of the larva, which marks off the oral cone from the bases of the tentacles, and just above the upper ends of the four endodermal folds this depression appears to be deeper. From the bottom of these deeper depressions, which are termed the **septal funnels**, ectoderm cells are budded off and force their way into the jelly between the limbs of the taeniolar folds. These cells develop fine muscular fibrils on their external surfaces, which form the four longitudinal septal muscles. These muscles extend down to the base of the larva; they are exceedingly irritable and serve to contract it (Fig. 49).

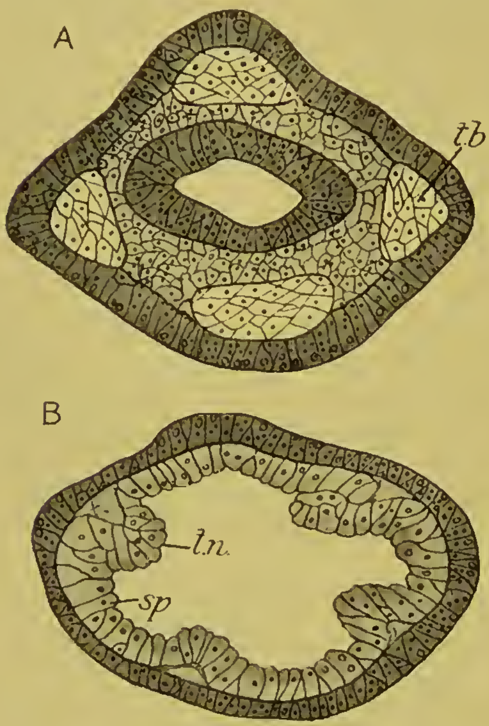

Fig. 48.—Two transverse sections through a Hydra-tuba with four tentacles. (After Hein.)

A, grazing the upper surface or oral disc. B, through the middle of the body. *s.p*, stomach pocket; *t.b*, endoderm cells forming the bases of the tentacle; *t.n*, taeniolae.

The larva which is now provided with a flattened upper surface or oral disc, with four long tentacles with solid axes, with four endodermal septa, and four ectodermal septal muscles, is termed a **Hydra-tuba** or **Scyphistoma**. Four secondary tentacles alternating with the first four are soon added, and eventually eight tertiary ones, alternating with the primary and secondary, so that in all sixteen are formed.

As Sir J. Dalyell (1847), the first discoverer of this larva showed, lateral buds like those of a true *Hydra* can be formed, which repeat the structure of the parent and eventually become detached, and stolons can grow out from the body wall just above the base, extending a short distance, and from them other hydra-tubae can be given off.

Friedemann (1902) takes up his account of the development where Hein left off. The eight-tentacled hydra-tuba grows in size as it captures more and more prey. Then eight new tentacles make their appearance alternating with the former, so that the animal now possesses sixteen tentacles. The number is then raised to twenty-four by the appearance of eight new ones, and with this number the hydra-tuba attains the maximum of its development. The nematocysts on the tentacles increase till, by their aggregation, they form warts and finally garland-like thickenings.

Meanwhile, whilst the number of tentacles is increasing from sixteen to twenty-four, other changes supervene. Four new and larger invaginations of the ectoderm of the oral disc make their appearance, just in the positions occupied by the old septal funnels. These are the rudiments of the four **sub-genital pits** of the adult. Just under the oral disc a hole, the **ostium,** appears in each taeniola, so that this structure is transformed from a complete ridge into a pillar, and the four gastric pouches of the hydra-tuba become in this way converted into the so-called **ring-sinus** (Fig. 51).

The free edge of the taeniola thickens and grows out into two diverging lips, which are the first rudiments of the **gastral filaments,** and these are covered in the adult with specially active digestive cells. From the oral side of the first eight tentacles there appear eight bud-like warts on the oral disc, which are the rudiments of the sense-organs of the adult. Beyond the ring-sinus eight lobes grow out; four of these, termed **per-radial,** are outpouchings of the original spaces between the taeniolae, whilst the other four, termed **inter-radial,** take their origin from those portions of the ring-sinus which have developed from the perforations in the taeniolae. Thus the oral disc becomes drawn out into eight lappets, and the tentacles are then thrown off. Each lappet contains one of the eight lobes which have grown out from the ring-sinus; the lappet is forked at its distal extremity, and in the re-entrant angle of each fork is the rudiment of the sense-tentacle.

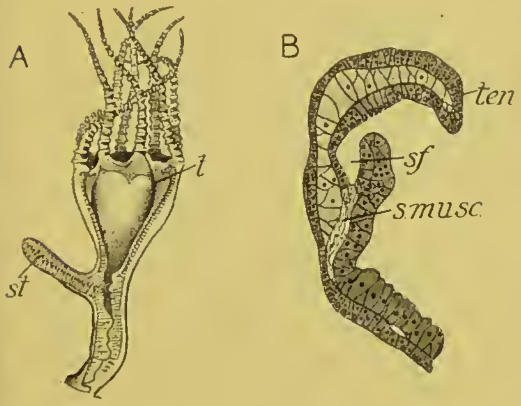

Fig. 49.—A, a Hydra-tuba with eight tentacles. (After Friedemann.) B, longitudinal section of a part of a similar specimen to show origin of septal funnels. (After Hein.)

s.f, septal funnel; *s.musc*, septal muscles; *st*, incipient stolon; *ten*, tentacle; *t*, taeniola.

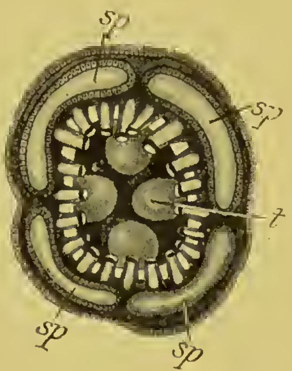

Fig. 50.—Oral view of Hydra-tuba with twenty tentacles. (The tentacles are represented as cut off.) (After Friedemann.)

sp, stomach pouch; *t*, taeniola.

The next process which occurs is the separation of the "head" or "crown" of the hydra-tuba from the stalk. This process is initiated by the appearance of a groove in the stalk; it can take place, as Friedemann shows, at different stages in the development, either before or after the loss of the tentacles.

If food is scarce the crown separates as a free-swimming organism termed an **Ephyra**, and the stalk slowly regenerates a new crown; but if food is abundant the process of the formation of a new crown begins before the old crown has separated, and before it is well under way a second groove appears below it, and a third crown starts to develop; and by a repetition of the process the Scyphistoma comes to look like a pile of plates, and is called a **Strobila**. This process is known as **strobilization**, and in this way one hydra-tuba can give rise to multitudes of Ephyrae (Figs. 52, 53).

The just liberated Ephyra is about an $\frac{1}{8}$ inch across the disc. The wart-like sense-tentacles develop otoliths in their distal endodermal cells, and this distal mass of endoderm becomes separated from the rest. The Ephyra does not attain the characters of the adult *Aurelia* until it has grown to a size of at least $\frac{1}{3}$ inch in diameter. The change in its shape, which brings in the adult features, consists in the slow growth of **adradial cushions**, which are situated between the bases of the eight lobes of the Ephyra. These cushions, by their growth, gradually fill up the deep re-entrant notches in the disc of the Ephyra, and change its star-like outline into the rounded outline of the adult *Aurelia*.

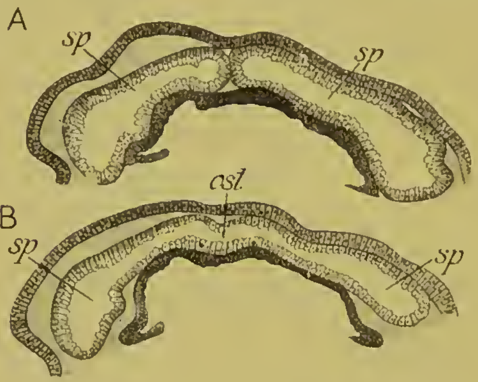

FIG. 51.—Two horizontal sections through the upper part of a Hydra-tuba, about as old as that represented in Fig. 50, to show the formation of the ostium connecting the stomach pockets. (After Friedemann.)

A, above the level of the ostium. B, at the level of the ostium; *ost*, ostium.

As each of these cushions grows, a new endodermal pouch grows out from the ring-sinus and extends into it. At the same time each endodermal pouch, which already occupies a lobe of the Ephyra, becomes trilobed at its distal extremity. The median branch of these three-pronged forks goes to the wart developed from the base of the hydra-tuba tentacle once situated there. This wart develops into the sense-tentacle of the *Aurelia* (Fig. 55). The two lateral branches of the forks go into the two folds forming the forked extremity of the Ephyra-lobe. These forks persist in the adult as the curtains which eventually form a hood for the sense organ of the adult. The oral cone becomes more and more prominent and forms the manubrium of the adult.

The genus *Pelagia* goes no farther than this stage. Each adradial cushion develops a single long tentacle, and one only. But in *Aurelia*, just as is the case with the Hydromedusan

Podocoryne (see p. 61), the endoderm of the radial pouches flattens out so as to form plates which meet each other in the interspace between two adjacent pouches, and thus form the so-called **cathammal plates**, or, collectively, the **endoderm lamella.** In these plates branches of the radial pouches hollow themselves out. Some of these branches, at right angles to the pouches, form a circular canal just as happens in *Podocoryne*; others form branches of the per-radial and inter-radial pouches. The last formed adradial pouches do not branch. The numerous small tentacles which fringe the disc of the adult, arise as sprouts from the circular canal (Fig. 56).

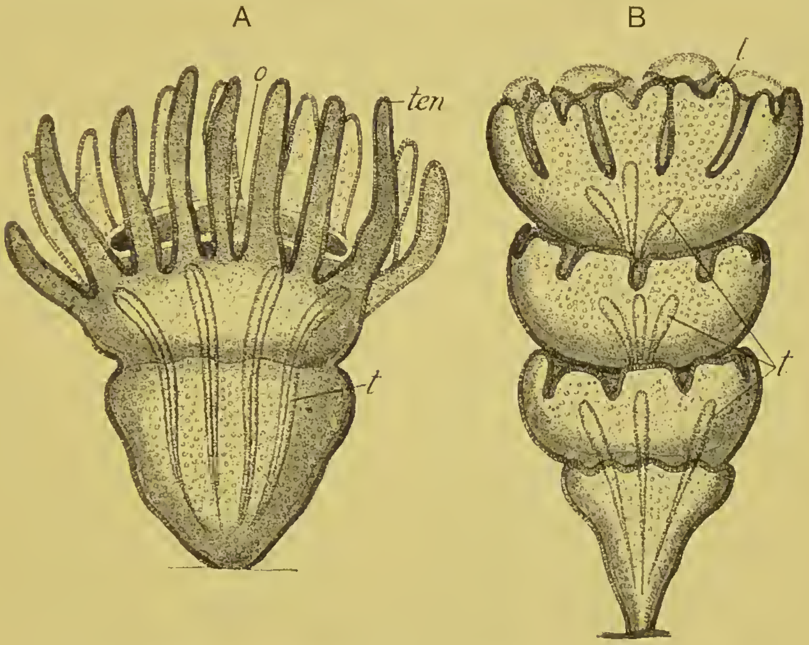

FIG. 52.—Two stages in the strobilization of the Scyphistoma of *Aurelia aurita*. (After Claus.)

A, the appearance of the first transverse groove. B, tentacles lost, four transverse grooves. *l*, lobe of Ephyra ; *o*, oral cone ; *t*, taeniola ; *ten*, tentacle.

OTHER SCYPHOZOA

Hein (1903) has investigated the early development of another genus *Cotylorhiza.* It agrees very closely with *Aurelia*, but the original blastopore closes, and the mouth is formed later by the reopening of this orifice. The temporary closure and subsequent reopening of an orifice is to be noted as a phenomenon of very frequent occurrence in development; we interpret it as a sign that the orifice in question is no longer continuously functional.

Pelagia is an instance of a form modified for oceanic life; it develops a blastula like that of *Aurelia*, and a gastrula is formed by invagination as in that animal; but the original blastocoele persists in the aboral end of the larva since the endodermic sac remains relatively small. This again is a feature which we find in many larvae which are adapted for continuous free-swimming life. The larva never fixes itself, and eventually the lobes of the Ephyra grow out in a circle round the mouth. The whole development is therefore modified along quite similar lines to those exhibited by the Geryonidae, the hydra-tuba being modified into a floating larva, just as is the hydroid stage of the Geryonidae.

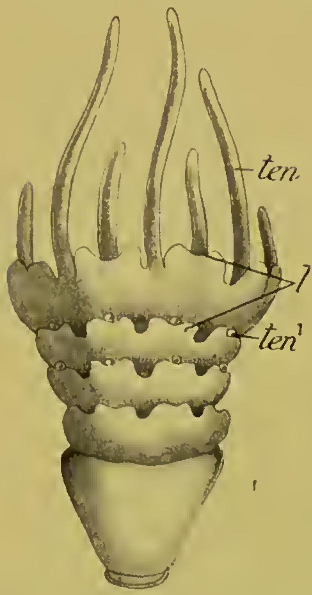

Fig. 53.—A strobilized Scyphistoma of *Aurelia aurita*. (After Claus.)

l, forked lappet of edge of disc of Ephyra; *ten*, tentacles of Hydra-tuba degenerating; *ten*¹, rudimentary sense-tentacles of Ephyra.

When we review what we have learned of the development of Scyphozoa, we are struck at first by the great differences between their life-histories and those of the Hydrozoa. A deeper and closer analysis tends, however, to diminish the supposed differences very much. It has been shown that many Hydroid colonies periodically lose and regenerate the "polyps" (Allman, 1871-2), (*i.e.* the swollen distal ends or heads of the hydroids which carry the tentacles and genital organs), that during the winter the polyps are often absent, and that these are regenerated from the basal stumps in

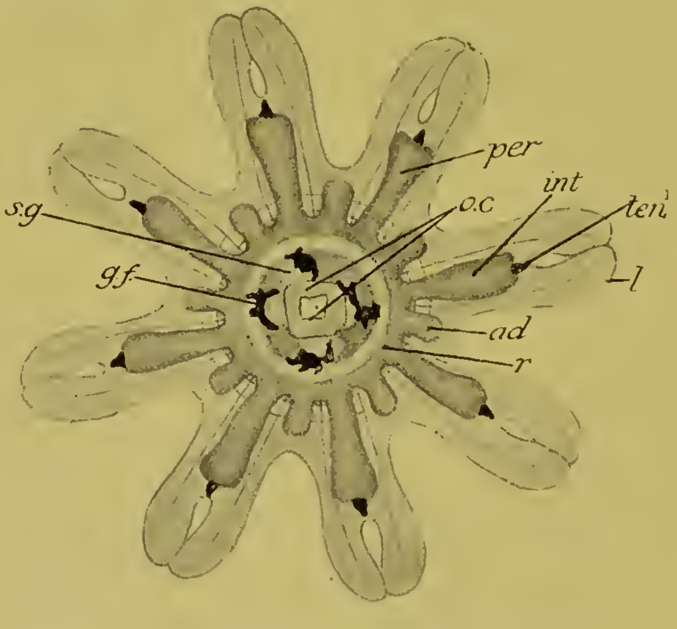

Fig. 54.—An Ephyra larva of *Aurelia aurita* just after liberation from the strobilized scyphistoma. (After Friedemann.)

ad, adradial lobe of ring-gut; *g.f*, gastral filament; *int*, inter-radial lobe of ring-sinus; *l*, forked lappet of edge of disc; *o.c*, oral cone; *per*, per-radial lobe of ring-sinus; *r*, ring-sinus; *sg*, sub-genital pit; *ten*¹, sense-tentacles.

the summer. This is very much the same phenomenon as the "strobilization" of the Scyphistoma, when the first "Ephyra-head" is budded off.

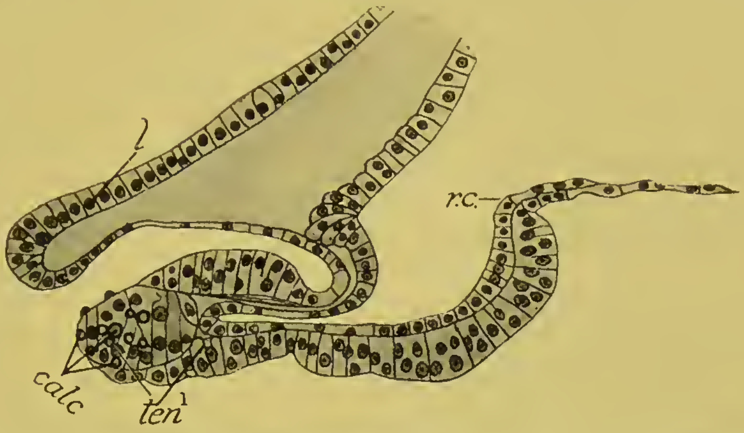

FIG. 55.—Longitudinal section through the sense organ of a young Ephyra.

calc, calcareous concretions in the sense-tentacle; *l*, lobe of disc of Ephyra; *r.c*, wall of radial canal; *ten*1, sense-tentacle.

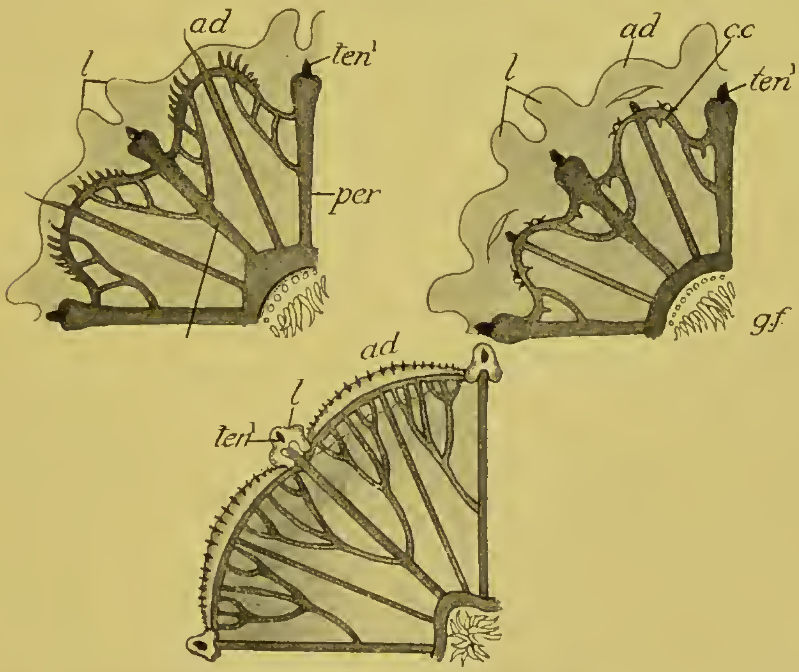

FIG. 56.—Three stages in the development of the Ephyra larva into the adult *Aurelia*. (After Claus.)

ad, adradial cushion; *c.c*, circular canal; *g.f*, gastral filament; *l*, lobe sheltering sense tentacle; *per*, per-radial canal; *ten*1, sense-tentacle. (The reference line without letter in the first figure points to an inter-radial canal.)

Then in both groups the sexually mature person is typically a person which breaks loose from the colony and develops a swimming-web. In Hydrozoa such a person breaks loose in such a way as to leave practically no stump, but in Scyphozoa a stump is left which can regenerate a new head. This is what the only difference, often much emphasized, between the formation of a medusa by lateral budding and the formation of a medusa by transverse fission consists in.

The fact that the sexual cells of Scyphozoa are discharged inwards towards the stomach cavity, and not outwards as in Hydrozoa, is a real difference. But it must be remembered that it is confidently asserted that in many Hydrozoa with rudimentary gonophores the sexual cells originate in the endoderm; and it may be that they always have an ectodermal origin, but that in their first stages they are indistinguishable from ectodermal interstitial cells. Gastral filaments and septal muscles constitute, however, features in which Scyphozoan organization is higher than that of Hydrozoa.

III. ACTINOZOA

The great group of the Actinozoa, one of the four primary groups of the Coelenterata, is distinguished from the Scyphozoa by the replacement of the oral cone by an inturned tube of ectoderm, the **stomodaeum**.

The eggs of Actinozoa, like those of Scyphozoa, are developed in the endoderm and dehisced into the coelenteron of the parent. In most Zoantharia the embryos pass through the first stages of their development within the body of the mother; but in a few Zoantharia and apparently in most Alcyonaria, the eggs are discharged through the mouth of the parent into the sea and fertilized there. In this case it is possible to obtain a great many specimens of the same age. But when development takes place within the coelenteron of the mother only a very few specimens of the earliest stages of development will be found in any one individual parent, since these stages are rapidly passed through. For this reason we select a Zoantharian (*Urticina crassicornis*), in which the eggs are discharged before fertilization, as a type for special study in order to illustrate the development of Actinozoa.

URTICINA CRASSICORNIS

Urticina crassicornis is a sea-anemone found on the British and Norwegian coasts and its development has been worked out by Appellöf (1900). This observer kept the adults living in tanks in the Bergen aquarium until they spawned; he kept the eggs in dishes of clean sea-water until the larvae hatched out, and these he was able to keep alive until they fixed themselves and metamorphosed into young sea-anemones. As preservative he used the mixture of corrosive sublimate solution and acetic acid described in Chapter II.

The egg of *Urticina* when discharged is surrounded by a gelatinous mass, through which presumably the fertilizing spermatozoon has to penetrate. After fertilization this gelatinous mass hardens into a firm capsule beset with spines, and within the shelter of this capsule the early stages of development are passed through. The egg is composed of a thin rind of relatively clear cytoplasm and an internal zone of cytoplasm loaded with large spheres of yolk. The kernel of the egg consists of a mass of material with sparse yolk spheres but with many granules, which appears to be reserve food material not cytoplasm.

The egg is thus of the type called **centrolecithal** in Chapter I., and its segmentation is a matter of considerable interest. The zygotic nucleus is situated in the outer clear layer of cytoplasm, and there it undergoes its first division. The daughter nuclei migrate into the deeper layer of yolky cytoplasm and here undergo repeated division until sixteen nuclei have been formed, which are distributed around the periphery of the egg in the yolky layer. Then and then only the cytoplasm begins to be divided into blastomeres, of which consequently sixteen are produced at once. At first (Fig. 57) the blastomeres are separated from one another only at their outer ends, but they soon become sharply defined over their whole surfaces; nevertheless, even when so defined, their inner ends are embedded in the mass of reserve material which forms the innermost core of the egg.

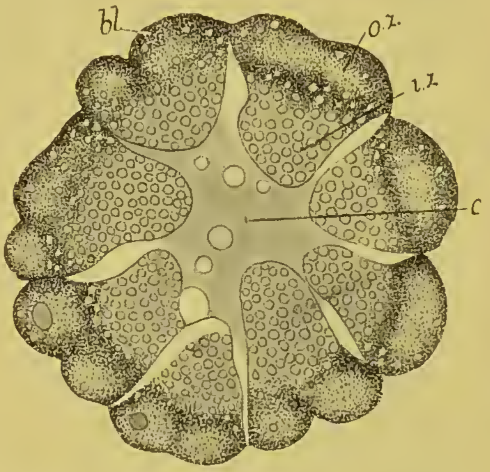

Fig. 57.—An egg of *Urticina crassicornis* dividing into sixteen blastomeres. (After Appellöf.)

bl, blastomeres; *c*, core of nutritive material in the centre of the egg; *i.z*, inner zone of yolky cytoplasm; *o.z*, outer zone of clear cytoplasm.

As segmentation proceeds a **blastula** is eventually formed, whose wall consists of a single layer of small cells, but in whose cavity there still remains the mass of "reserve-material" which formed the kernel of the unsegmented egg (Fig. 58).

The blastula is at first ellipsoidal, but one pole becomes flattened and in the centre of this pole an invagination takes place. The manner in which this occurs is of great interest. The borders of the patch which is to be invaginated bend in first, so that for a brief period its central part projects like a knob. As the process of invagination proceeds the centre is also carried downwards and inwards, and thus a two-layered organism—*i.e.* a **gastrula**, is produced. Occasionally the reserve material in the blastocoele persists in considerable quantity; it adheres to the centre of the area which is normally invaginated and thus impedes the process of invagination.

This plug which normally persists for only a short time, persists in this case much longer, and eventually the cells forming the borders of the area of invagination are carried in past it, so that the invaginated layer, or endoderm, is represented by a solid plug of material surrounded by a layer of cells. This plug is gradually digested by the surrounding cells and thus the hollow gastrula stage is reached.

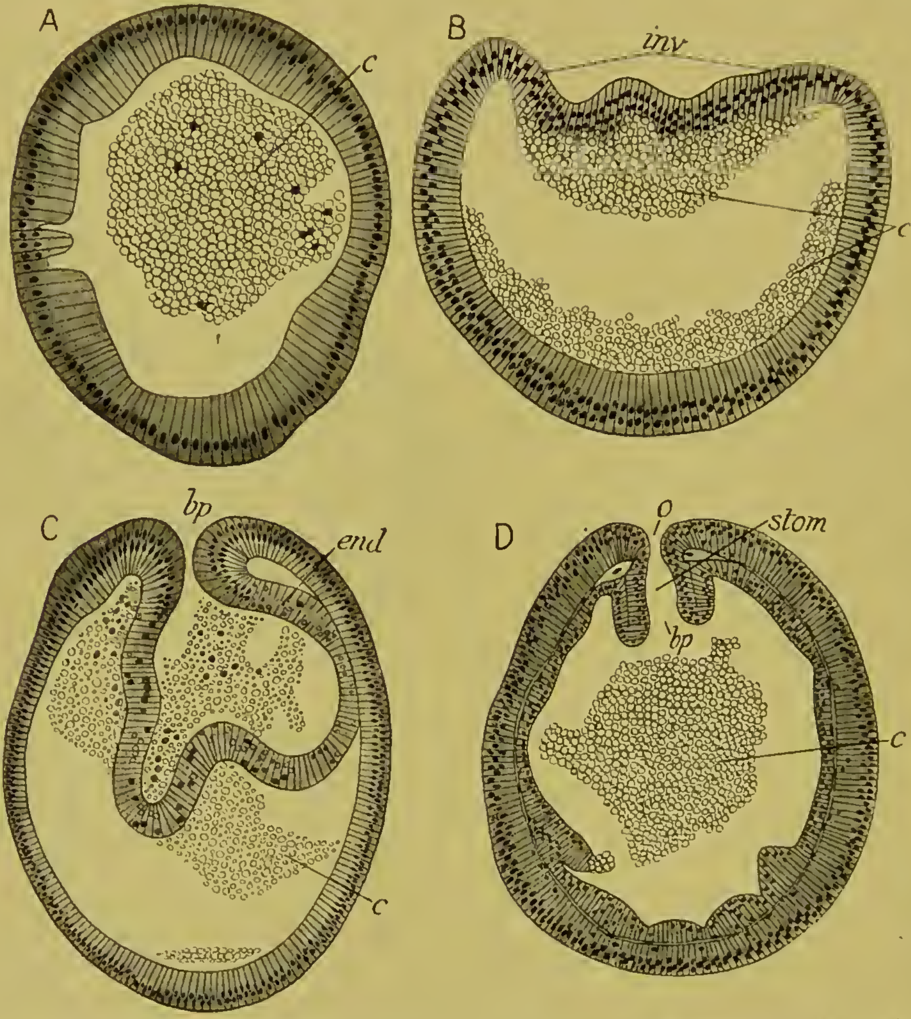

Fig. 58.—Four stages in the development of the egg of *Urticina crassicornis* as seen in longitudinal sections. (After Appellöf.)

A, blastula. B, blastula preparing to undergo invagination. C, invagination nearly complete, gastrula stage. D, formation of stomodaeum *bp*, blastopore ; *c*, remnants of the core of the egg ; *end*, endoderm ; *inv*, area of invagination ; *o*, mouth ; *stom*, stomodaeum.

The relation between the normal and abnormal methods of reaching the two-layered stage in this species is the same as the relation between the method of forming the planula larva in Scyphozoa and that in Hydrozoa. We may regard them as two varieties of **gastrulation**. The invaginated cells or endoderm, form an inner sac which spreads till it completely displaces the food material in the blastocoele, which is used up in nourishing the growing cells. Both ectoderm and endoderm then co-operate in producing a gelatinous secretion, the so-called jelly, **mesogloea,** or **supporting lamella.** The cavity of the gastrula becomes the **coelenteron** of the adult.

When the opening of the invagination has become narrowed so as to form a slit-like blastopore, the ectoderm acquires cilia and the embryo rotates within the egg-capsule, which shrinks and becomes more transparent. A little later the embryo escapes from the capsule altogether and swims about as a larva, but the swimming is not very vigorous and the larvae do not rise far from the bottom.

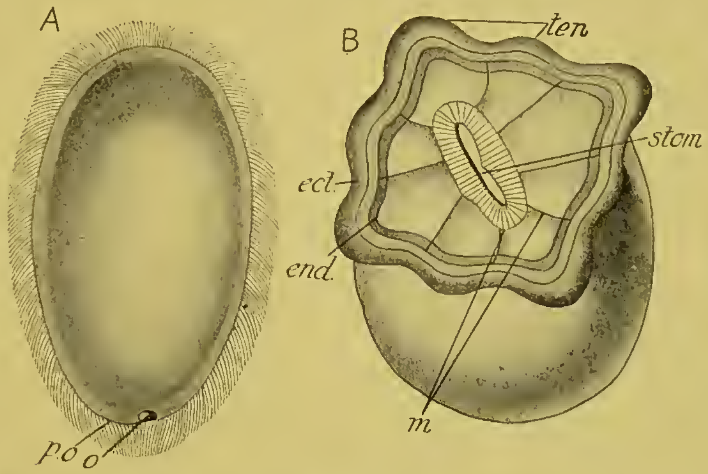

FIG. 59.—Two stages in the development of the larva of *Urticina crassicornis.* (After Appellöf.)

A, free-swimming larva. B, stage just before fixation. *ect*, ectoderm; *end*, endoderm; *m*, mesenteries; *p.o*, post-oral region; *o*, mouth; *stom*, stomodaeum; *ten*, tentacles.

The larvae are of ovoid shape with a broad aboral and a pointed oral end. The aboral end is directed forwards in swimming, but the reduced **blastopore,** which persists as the **mouth,** is not situated actually at the oral end but a little to one side of it, so that there is a small **post-oral projection** of the body. The ectodermal lips of the blastopore grow inwards and form an inwardly-projecting tube which is the **stomodaeum** (Fig. 58, D).

The **mesenteries** now make their appearance. The eight so-called primary ones are formed about the same time. Each originates as a fold of the endoderm which projects inwards into the gastric cavity: the cavity between the limbs of the fold being occupied by a layer of supporting lamella secreted by the cells forming the fold. The mesenteries correspond exactly to the **taeniolae** which occur in the Hydra-tuba of the Scyphozoa, and which are irregularly developed even in the polyps of the Hydrozoa. The eight mesenteries are arranged in four pairs, or, as it is usual to term them in this case,

couples. Of these, one couple, which project into the post-oral prominence of the larva, are known as the **dorsal couple**; then follow **dorso-lateral, ventro-lateral** and **ventral couples.** The ventro-lateral couple develop more quickly than the others, they soon reach the stomodaeal wall with which they fuse, and thus divide the gastric cavity into a dorsal and a ventral chamber. They also extend farther towards the aboral pole of the larva than the others, but as development proceeds all eight fuse with the stomodaeum and reach equally far towards the aboral pole.

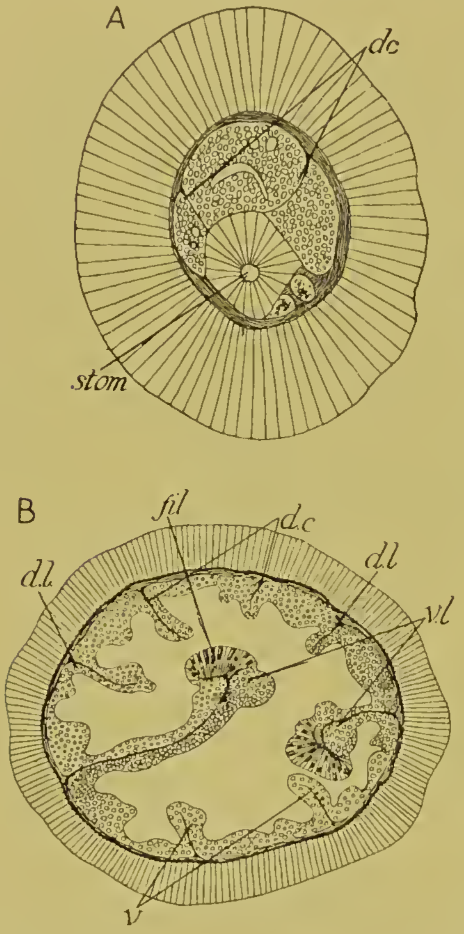

Fig. 60.—Two transverse sections through a larva of *Urticina crassicornis* to show the formation of mesenteries. (After Appellöf.)

A, section through post-oral region of larva. B, section through middle of larva. *d.c*, dorsal couple of mesenteries; *d.l*, dorso-lateral mesenteries; *fil*, mesenterial filament; *v.l*, ventro-lateral mesenteries; *v*, ventral mesenteries.

The chambers into which the coelenteron is divided by the eight mesenteries are arranged as follows. The **dorsal** chamber is a median chamber at one end of the long slit-like stomodaeum; then follow a pair of **dorso-lateral** chambers, then a pair of **lateral chambers,** then a pair of **ventro-lateral** chambers, and finally a median ventral chamber, making eight in all.

When the process of formation of mesenteries is complete, perforations take place in the septa, forming the so-called **mesenterial stomata,** and thus the cavities into which the coelenteron is divided by the mesenteries are placed in communication with each other. In *Urticina* there are two sets of these stomata, an inner set near the stomodaeum and an outer set near the outer body wall of the larva. Both sets arise in the same way; the supporting lamella becomes absorbed over a limited area and the two layers of cells which form the mesenterial fold fuse with one another, and then in the centre of this area of fusion absorption of the cytoplasm begins, and so a perforation is made (Fig. 61). We may note that the taeniolae of the Hydra-tuba larva become perforated in exactly the same way.

After swimming for about six weeks the larvae begin to attach themselves to the substratum by the aboral pole; at first the attachment is only temporary, it is some considerable time afterwards that a permanent attachment is effected and the **pedal disc** of the adult formed. Before this occurs the rudiments of the first eight tentacles make their appearance. Each arises as a simple outpouching of one of the eight chambers into which the coelenteron is divided (Fig. 59, B), and all appear to arise about the same time; those belonging to the dorsal and ventral chambers and to the lateral chambers are larger than those belonging to the other four chambers.

The thickened edges of the mesenteries, where they end freely in the coelenteron below the stomodaeum, are known as the **mesenterial filaments.** In the adult *Urticina* each filament is composed of a median strip of cells containing gland cells and **cnidoblasts**, flanked by two strips of cells carrying long cilia. The mesenterial filaments appear on the ventro-lateral mesenteries long before they appear on the others. They first appear in the neighbourhood of the stomodaeum and grow downwards as simple streaks of columnar epithelium; then the central portion shows the glandular modification of its cells, and much later the lateral portions develop cilia. The other filaments make their appearance in the same manner much later, when tentacles have already been developed. According to Appellöf, and here he has the support of other authors such as Gardiner (1902), the filaments are composed of ectoderm which has grown down from the stomodaeum.

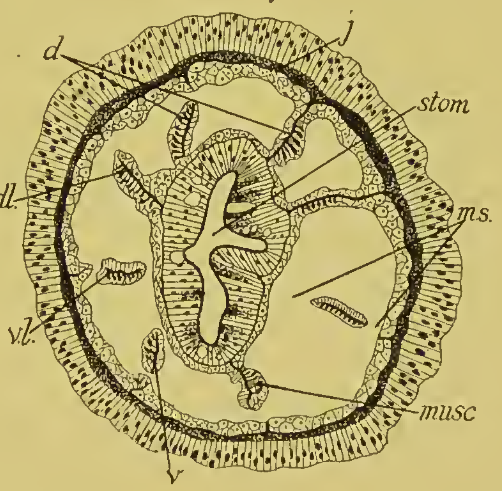

FIG. 61.—Transverse section through a larva of *Urticina crassicornis* in which the tentacles have just been developed. (After Appellöf.)

Letters as in preceding figures. In addition, *j*, jelly or supporting lamella; *m.s*, mesenterial stomata; *musc*, muscular thickening on mesentery.

In Alcyonaria where, as in the young *Urticina*, there are eight mesenteries, two only of these, the so-called dorsal mesenteries, bear filaments which carry cilia,—the other filaments being purely glandular in character whilst the ciliated filaments are devoid of gland cells. Following Wilson (1883), who is the best authority on Alcyonarian development, the two dorsal filaments are usually said to be of ectodermal origin whilst the others are stated to be endodermal, but the evidence which he adduces in favour of this view is, however, neither thorough nor convincing.

As the filaments are stated to close round the body of ingested prey and to form a sort of temporary alimentary tube within the coelenteron, and as their cells are stated to be the cells which secrete the digestive ferment, one would at first sight naturally expect them to be of endodermal origin. It is distinctly stated that in Alcyonaria the two dorsal ciliated filaments do not take part in the process of digestion, and if they are of ectodermal origin this is also what one would expect. But if we accept Appellöf's account of the development of the filaments in Zoantharia, we must suppose that the ectoderm in these animals has acquired digestive functions. Since in all groups of the animal kingdom which have been carefully examined, the distinction between protective ectoderm and digestive endoderm is the first physiological differentiation to be established, and the most deeply rooted, one would imagine it is unlikely that Zoantharia should in this respect form a solitary exception.

But some observations by Miss Pratt on the digestion of Alcyonaria (1905), appear to us to place the whole matter in a new light. She finds that gland cells similar to those found in the six ventral digestive filaments are found also in the stomodaeum, and that the tissues in these filaments are so similar to the stomodeal tissue that she believes that in Alcyonaria as in Zoantharia all the filaments originate from the ectoderm. The effect of the digestive juice is not to dissolve the bodies of the prey, but to break them up into granules, which are then ingested whole by the **endoderm** cells covering the mesentery beneath the filament. The cells forming the filament do not ingest anything. It thus appears that this so-called ectodermal digestion is merely a preparatory process similar to that exercised by salivary glands in Mammalia, which, like the filaments, are of ectodermal origin, and that in all cases the final digestion and the assimilation are functions performed by the endoderm alone.

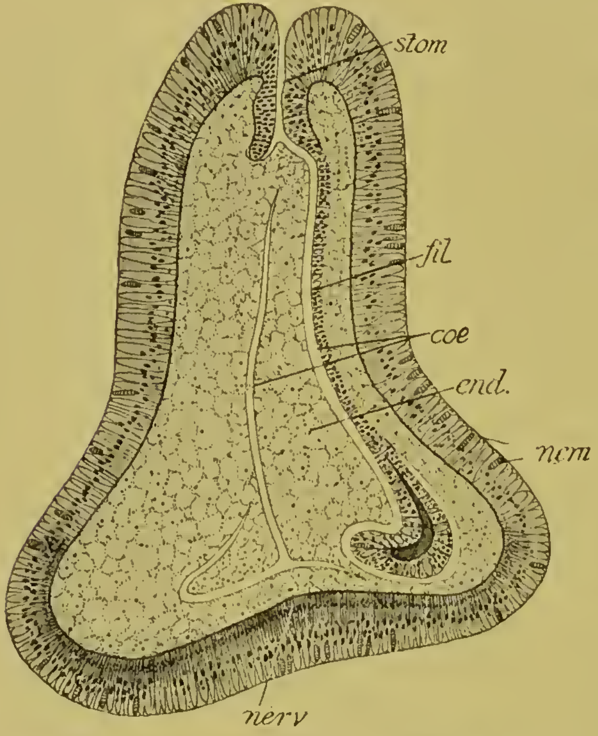

FIG. 62.—Longitudinal section through the larva of *Agaricia agaricites* to show the ectodermal origin of the mesenterial filament. (After Duerden.)

coe, coelenteron ; *end*, endoderm ; *fil*, mesenterial filament ; *nerv*, nervous tissue at the aboral end of larva ; *ncm*, nematocyst.

The muscular thickenings which run along the faces of the mesenterics appear about the same time as the tentacles. The supporting lamella becomes beset with branches on one side which are secreted by the overlying endoderm cells, these increase in number and develop basal longitudinal muscular tails. The muscles first appear on the ventro-lateral mesenteries, and afterwards on the others. Those on the ventral mesenteries are turned away from one another, and those on all the other mesenteries are developed on that side of each mesentery which faces towards the ventral mesentery of its own side. It follows that the muscle thickenings on the dorsal mesenteries are also turned away from one another.

The dorsal and ventral couples which are attached to the ends of the stomodaeum are known as **directive mesenteries.** In many Actinozoa the ends of the stomodaeum are developed into strongly ciliated grooves—the so-called **siphonoglyphes** or **gonidial** grooves. In practically all, including all Alcyonaria, one end of the stomodaeum is thus modified; this is called the ventral end, and to this end the ventral directive mesenteries are attached.

The arrangement of muscle thickenings just described is the same as that which persists for life in the family Edwardsiae, in which family also, as in the young *Urticina*, there are only eight fully formed mesenteries and eight tentacles. *Urticina* therefore passes through an "Edwardsia" stage in development, and this has been proved to be true of every Hexactinian whose development has been worked out.

In the very oldest specimens of *Urticina* which Appellöf was able to rear he found that extra mesenteries were being developed. These extra mesenteries consist of two on each side, four in all, and each of the new mesenteries was in such a position as to make a pair with a ventro-lateral or lateral mesentery, and each bore a muscle thickening facing the muscle of its fellow in the pair (see Fig. 67). Such a **pair,** consisting of two mesenteries facing one another, is to be carefully discriminated from a **couple,** the two mesenteries forming which are situated at symmetrical points on opposite sides of the stomodaeum.

Since the dorsal and the ventral couples of mesenteries may also be regarded as forming two pairs, we reach in this way a total of six pairs of principal mesenteries, and this is what is known as the typical Hexactinian arrangement, the most widely distributed arrangement amongst Zoantharia. The powerful sphincter muscle which in the adult closes the mouth was represented in the oldest of the artificially-reared specimens merely by a thin sheet of circular fibres.

Appellöf has also examined the development of a species of the commonest genus of sea-anemones, *Actinia*. In this species, however (*Actinia equina*), the earlier part of the development is passed through in the coelenteron of the mother, and Appellöf was unable to obtain a complete series of stages of this form; however, one or two points of interest were made out.

The endoderm originates by proliferation from the ectoderm cells; a proliferation probably confined to one pole. A hollow two-layered planula larva is formed which is devoid of a mouth; but a mouth is formed later by the fusion of the two layers of the body wall at the oral pole and their perforation at this point.

The larva possesses an anterior wisp of long cilia, the cells carrying which are excessively attenuated and have all the appearance of sense cells; and at their bases are a few rounded cells with tails, obviously ganglion cells. In the larvae of *Agaricia* according to Duerden (1902), although there is no bunch of long cilia at the aboral pole, there is at this spot a comparatively thick layer of nerve fibres (Fig 62).

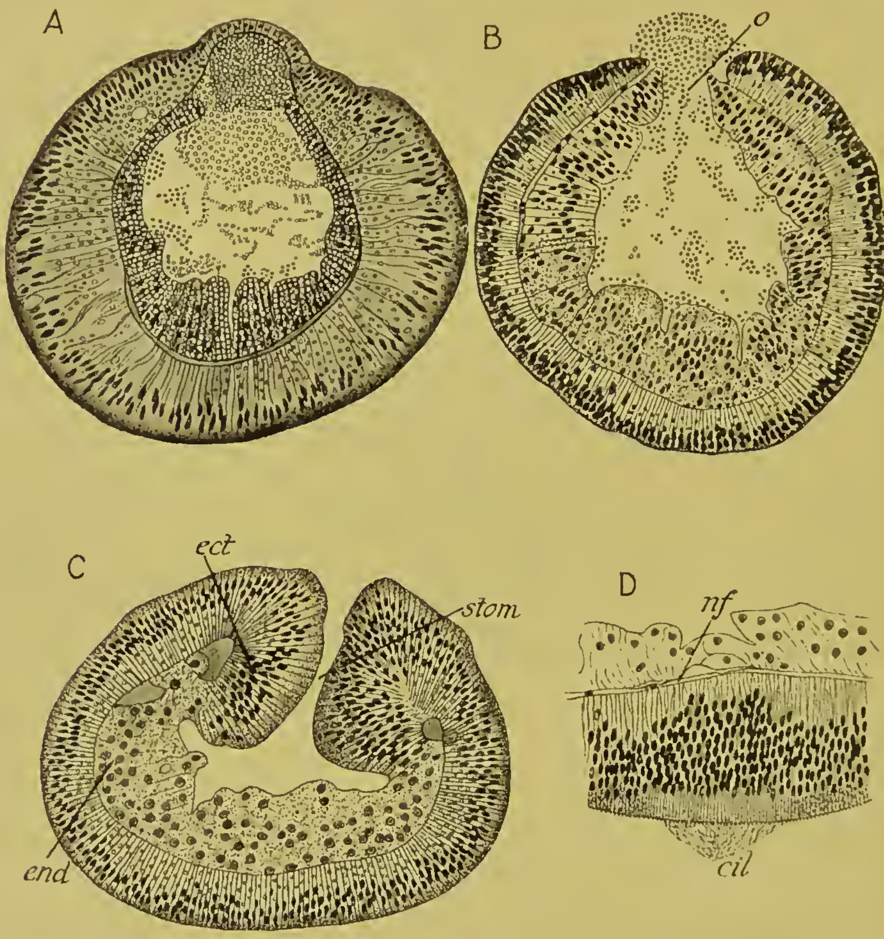

FIG. 63—Stages in the development of the larva of *Actinia equina*. (After Appellöf.)

A, stage just before the formation of the mouth, the endoderm is already absorbed over the spot where the mouth will be formed. B, stage when the mouth is formed. C, stage after formation of stomodaeum. D, aboral pole of free-swimming larva much enlarged. *cil*, long aboral cilia; *n.f*, nerve fibres. Other letters as before.

In the species *Actinia bermudensis*, the development of which has been worked out by Cary (1910), although the egg passes through the earlier stages of development within the coelenteron of the mother, yet the endoderm appears to be formed by invagination just as in *Urticina crassicornis*. On the other hand, in the species *Metridium marginatum* which has been examined by McMurrich (1891), and *Sagartia parasitica* and *Adamsia palliata*, which have been examined by Faurot (1903), although the eggs are expelled from the mother previous to fertilization yet the endoderm appears to be formed by proliferation from the outer cells of the embryo, that is, from cells which become the ectoderm; and in the two latter species examined by Faurot this occurs at a very early period of development. On the whole this method of forming the endoderm appears to be much commoner than the method of invagination among Zoantharia, and it is the only method recorded for Alcyonaria. In many cases the endoderm cells are so swollen that, when the mesenteries appear, the whole coelenteron is choked up by these cells and only slit-like remnants of the cavity of the coelenteron remain.

The two families of Cereanthidae and Zoanthidae are formally classed along with the Hexactiniae as Zoantharia, but they exhibit a very different arrangement of mesenteries and in each case the egg develops into a most characteristic larva. A good summary of what is known about the larvae of both families is given by Carlgren (1906).

The larva of the Cereanthidae is termed **Arachnactis**, and it is characterized by the excessive prolongation of the free-swimming stage. Two sets of tentacles, an inner and an outer, and numerous mesenteries are developed whilst the larva still continues to swim. Of these mesenteries, there is a ventral pair of "directives" attached to one end of the stomodaeum, and two "couples" attached to its sides. The space which should be occupied by the dorsal directives is at first empty, but young mesenteries appear in it later, varying in number with the age of the larva. They are formed alternately to the right and to the left of the median line, and at first they are short, only later reaching the stomodaeal wall.

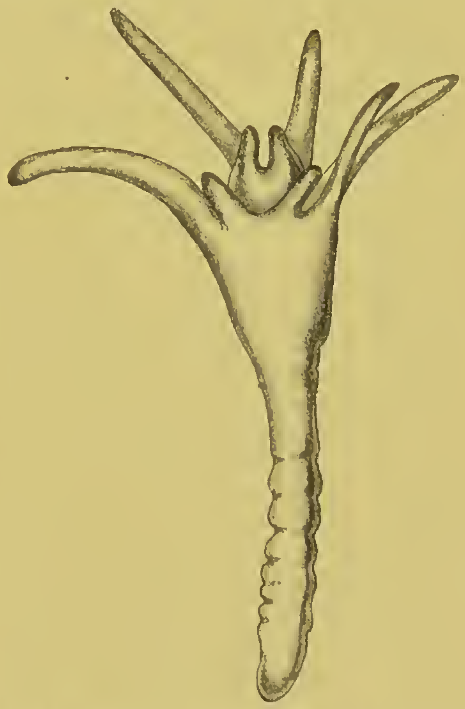

FIG. 64.—The Arachnactis larva of *Cereanthus membranaceus*. (After Carlgren.)

The larva of the Zoanthidae appears under two varieties, in one of which (**Zoanthella**) there is a transverse girdle of strong cilia, whilst in the other (**Zoanthina**) there is a longitudinal band of cilia as locomotor organ. It develops no tentacles until twelve mesenteries have been formed, and then the twelve are developed in a single cycle. The twelve mesenteries are arranged in six pairs which correspond to the six pairs of Hexactiniae, but the dorsal "directives" are short and do not reach the stomodaeum, and one mesentery of each of the lateral and ventro-lateral pairs remains short, whilst its fellow is long and joins the stomodaeum. In the space which intervenes between the ventral "directives" and the ventro-lateral pair on each side, there is a growing zone where new pairs of mesenteries are added, one fellow of each new pair being long and one short.

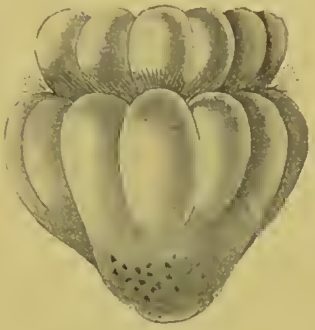

FIG. 65.—The Zoanthina larva of a Zoanthid. (After Carlgren.)

It appears from this brief review that the larvae of Cereanthidae, unlike the Hexactiniae, do not pass through an "Edwardsia" stage in their development, but that nevertheless they may be regarded as springing from a stock, common to the Hexactiniae, Edwardsiae, and themselves, in which only six mesenteries were developed. The Zoanthidae on the contrary may almost be said to pass through a Hexactinian stage in their development.

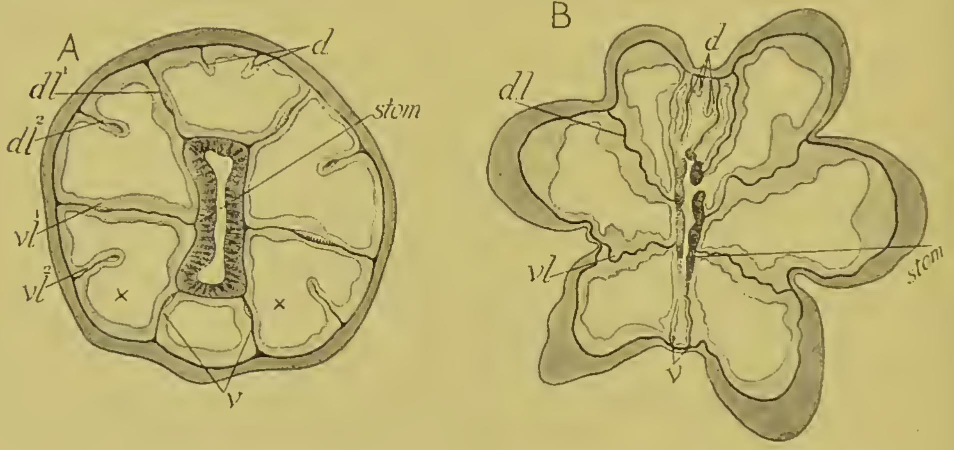

FIG. 66.—Transverse sections of Actinozoan larvae. (After van Beneden.)

A, section of Zoanthina larva. B, section of Arachnactis larva. *d*, (in A) dorsal directive mesenteries, (in B) indefinite number of mesenteries occupying the place of the dorsal directives; *d.l*, dorso-lateral mesenteries; $d.l^1$, long dorso-lateral mesenteries; $d.l^2$, short dorso-lateral mesenteries: *v*, ventral directive mesenteries; *v.l*, ventro-lateral mesenteries; $v.l^1$, long ventro-lateral mesenteries; $v.l^2$, short ventro-lateral mesenteries; *xx* (in A) mark the places where additional pairs of mesenteries are added.

We have much less information on the development of Alcyonaria than on that of Zoantharia. The best account is that of Wilson, to which the more recent accounts by Koch of the development of *Gorgonia* (1887), and by Hickson of the development of *Alcyonium*

(1901), have added nothing of any importance. Wilson worked on the species *Renilla reniformis* and *Leptogorgia virgulata* (1883). The eggs are expelled from the parent colonies and fertilized in the sea. The endoderm appears to be formed by proliferation, and all the eight mesenteries appear at once. The arrangement of the muscular thickenings on these mesenteries differs only from that in the Edwardsia stage of Hexactiniae, in the fact that the thickenings of the ventral directives face each other instead of being turned away from each other. Wilson's observations on the origin of the filaments have already been dealt with.

The Alcyonaria differ less in this respect from the Edwardsiae than these differ from the Cereanthidae, and the real ground of their separation from the Zoantharia lies in the method of forming the skeleton, as will now be made clear.

The popular name Sea-Anemone is usually given to those Zoantharia which do not develop a calcareous skeleton, whilst those which do form skeletons are termed Coral-forming Polyps — or briefly, Corals. The development of the skeleton in these was first worked out by Lacaze-Duthiers (1864), but the subject was again taken up and thoroughly examined by von Koch (1897), whose latest investigations deal with the Mediterranean species *Caryophyllia cyathus*.

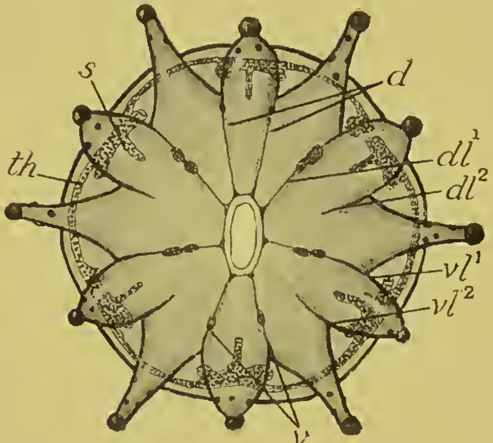

Fig. 67. — Young living *Caryophyllia cyathus* seen from above. The calcareous skeleton shows through the transparent tissue. (After von Koch.)

Letters as in preceding figures. In addition, *s*, one of the primary septa; *th*, wall of theca.

The first part of the skeleton to appear is the **basal plate** which is secreted by the pedal disc of the polyp. This basal plate appears as six separate areas of calcareous deposit, one area being situated beneath the space intervening between each pair of mesenteries. They eventually coalesce to form, first a six-rayed star and then a circular disc. In the centre of each of the original areas the process of secretion of calcareous matter continues more actively than elsewhere, and the consequence of this is the formation of six radiating **septa** of calcareous matter, each septum being covered by an inwardly projecting fold of the pedal disc. The edge of the basal plate becomes raised into a rim, owing to the upward extension of the skeleton-forming area on the side of the polyp, and in this way the beginning is made of a **theca** or cup in which the polyp sits.

Both septa and thecal wall grow in height: soon a set of six secondary septa alternating with the primary ones make their appearance, whilst in the centre of the basal plate there appear two or three small knobs which will later coalesce so as to form the **columella**. The columella is the median pillar which projects upwards from the base of the theca, indenting the base of the polyp. The primary septa become extremely thick where they join the thecal wall. Still later, cycles of tertiary septa make their appearance, and from the edges of these structures isolated pillars become separated which form the **pali**.

The skeleton of Zoantharian corals is therefore purely derived from an external exudation, and in this respect it contrasts most markedly with the skeleton of Alcyonaria. In his paper on *Renilla* (1883), Wilson has described the origin of a typical Alcyonarian skeleton. The lower cells of the ectoderm, corresponding roughly in position to the interstitial cells of *Hydra*, acquire calcareous concretions in their interior and migrate into the jelly. These concretions form the characteristic Alcyonarian spicules. But in *Renilla*, oval, wine-red, calcareous bodies are also formed in endodermal cells, and both kinds of spicules are found in the adult. In their characteristic skeleton therefore, as has already been said, lies the real distinguishing mark of Alcyonaria.

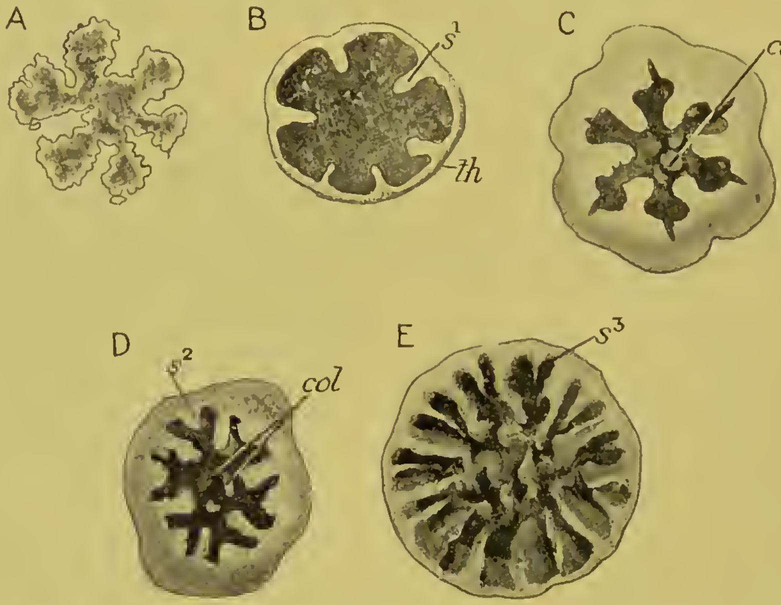

FIG. 68.—Five stages in the development of the skeleton of *Caryophyllia cyathus*. (After von Koch.)

A, the six primary areas of deposit coalescing to form a star-shaped figure. B, the thecal wall just formed. C, the thecal wall thickened opposite the bases of the septa. D, the appearance of the secondary septa and of the columella. E, tertiary septa formed. *col*, columella; s^1, primary septum; s^2, secondary septum; s^3, tertiary septum; *th*, wall of theca.

IV. CTENOPHORA

The fourth group of Coelenterata differ profoundly from all the rest, not only in their completely pelagic life, with no trace of a fixed stage anywhere in their ontogeny, but also in the possession of a well-developed nervous and sensory centre at the aboral pole, a part of the body which in other free-swimming Coelenterata is the least sensitive portion of the whole surface. The whole development is also of a widely different type from that of other Coelenterata, so that at first sight it is difficult to find any points of resemblance.

The most primitive type of Ctenophore known is that included in the order Cydippidea. Unfortunately such forms cannot be regularly obtained; occasionally they turn up in great swarms, and then for years it will be difficult if not impossible to procure them. On the other hand *Beröe* is a form which is abnormal in many respects, but which can be obtained regularly in the Mediterranean, at least at one season of the year, and it has been made the subject of much experimental work. Two species of *Beröe* occur in the Mediterranean, *B. forskalii* and *B. ovata.* The development of these two seems to be identical for all practical purposes and our illustrations will be drawn from each.

BERÖE

Beröe differs from primitive Ctenophora in possessing an enormously expanded stomodaeum, recalling the cavity of the bell of a medusa, and also in not having any vestige of tentacles.

We owe our first account of the development of *Beröe* to Chun (1880), and his account has been supplemented by those of Driesch (1895), Ziegler (1898), and Fischel (1897 and 1898), all of whom approached the subject from the standpoint of Experimental Embryology.

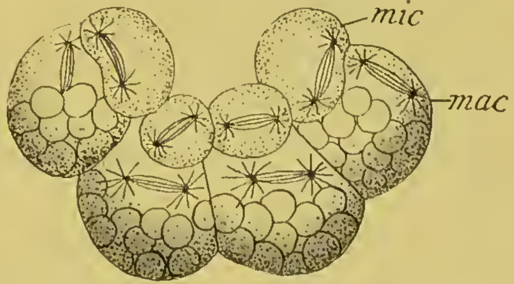

FIG. 69.—Side view of the segmenting egg of a Ctenophore (*Callianira bialata*). (After Metschnikoff.)

Only one half of the egg is seen; it is in the 16-cell stage. *mac*, macromere; *mic*, micromere.

If *Beröe* be kept in an aquarium it will deposit its ripe eggs. These are translucent spheres about 1 mm. in diameter, covered by a tough membrane. They contain a large amount of food-yolk. The nucleus is situated near one pole (the upper), and at this pole the most of the protoplasm of the egg is massed, the rest of the egg consisting of food-yolk.

Development goes on within the membrane up to the formation of a complete larva, and owing to the transparency of the eggs the greater part of the development can be studied in the living object; but larvae can be preserved in osmium acid and embedded in celloidin and then examined by cutting them into series of sections. The egg divides into two, and then four equal segments, by means of furrows which begin at the upper pole and slowly make their way through the yolky portion of the egg. The four segments then divide into eight, not as one finds in

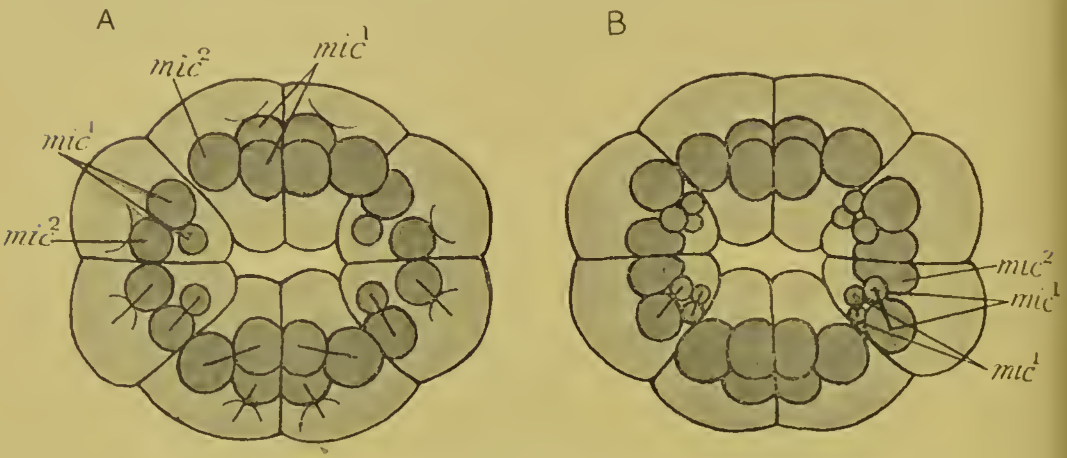

FIG. 70.—Two views of the developing egg of *Beröe ovata* seen from above. (After Ziegler.)

A, the first-formed micromeres have just divided and a second micromere has been budded off from each macromere. B, the daughters of the first micromeres have divided again. *mic*^{1}, daughters of first-formed micromeres; *mic*^{2}, micromeres budded off subsequently.

other eggs by a circular furrow, but by oblique almost vertical furrows, which separate off four inner larger cells from four outer smaller cells.

The eight cells are arranged in two linear rows of four each. These rows stand opposite to one another and form an ellipse-like

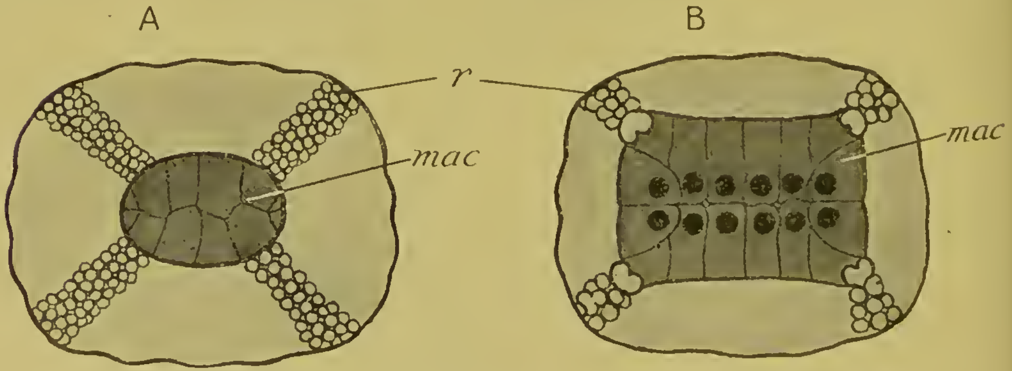

FIG. 71.—Oral and aboral views of the embryo of *Beröe ovata* in a later stage. (After Ziegler.)

A, from aboral pole. B, from oral pole. *mac*, macromeres; *r*, small ectoderm cells which later will develop into the ribs of the adult.

figure, the long axis of which is at right angles to the stomach plane of the adult, and is identical with the funnel plane of the adult Ctenophore. These eight cells are termed the **macromeres**. Then each macromere, by unequal division, buds off a much smaller cell termed a **micromere** at the upper pole, and thus a 16-cell stage is reached.

Now Ziegler (1898) has shown that the process of division of a blastomere takes nearly an hour to accomplish. During the process of the division of the eight blastomeres into two tiers of cells, the cytoplasm flows from the daughter cell, which is originally the larger, upwards into the smaller cell; so, by the time the division is accomplished the original proportions of the two cells have become reversed, so that what was originally macromere is now micromere and *vice versa.*

At the next cleavage eight more micromeres are budded off; this is the division of the macromeres of the 16-cell stage; and the first-formed micromeres divide, and thus the cleavage is complete, every cell in the egg having divided, and a total of thirty-two cells has been reached. Each macromere with the micromeres to which it gives rise, may be termed an **octant** of the egg. In Fig. 69 the egg of another Ctenophore is shown in this stage of development, seen from the side. All Ctenophora, the development of which has been examined, seem to agree in the way in which the cleavage of the egg is carried out, and this figure may therefore be taken as representing what goes on in *Beröe.*

In the outer octants the division of the first-formed micromeres is unequal, the smaller daughter cell being the smallest of all three sets of micromeres, and the larger daughter the largest of them; the new micromeres which have just been budded from the macromeres being intermediate in size. In the middle octants the first-formed micromeres divide equally.

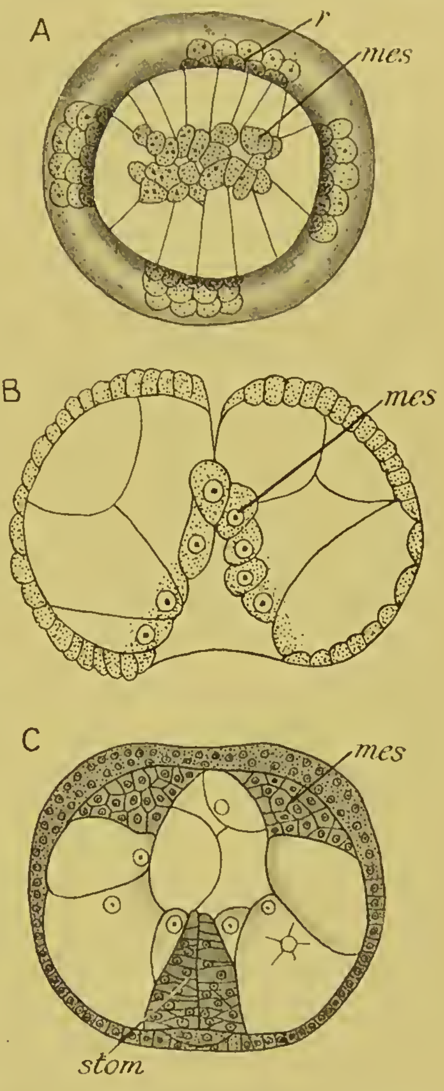

Fig. 72.—Illustrating the origin and fate of the so-called mesoderm in a Ctenophore embryo (*Collianira bialata*). (After Metschnikoff.)

A, view from oral pole at the time when the "mesoderm" is being budded off. B, optical longitudinal section of a slightly later stage, showing rotation of the macromeres and the consequent invagination of the "mesoderm." C, optical section of a still later stage, showing accumulation of mesoderm at upper pole and formation of stomodaeum. Letters as before. *mes*, mesoderm.

In the next period of cleavage the micromeres alone divide, the macromeres remaining quiescent. The smallest micromere divides into two equal parts. Its sister cell separates off towards the upper pole as a smallest tertiary micromere, and, as all the daughters of the first-formed micromeres in the middle octants likewise throw off a smallest micromere, there arises in this way a crown of smallest micromeres surrounding one pole of the embryo.

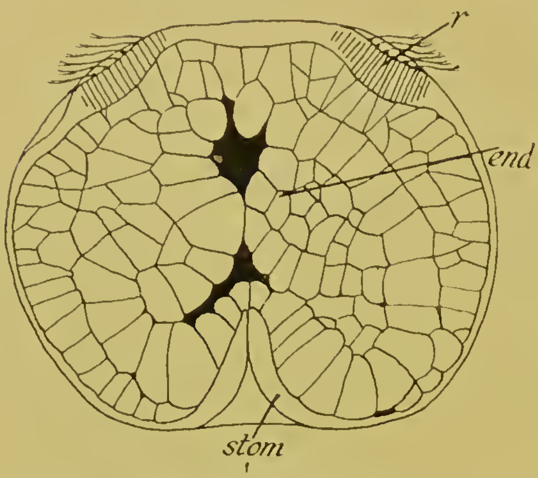

FIG. 73.—Optical section of embryo of *Beröe forskalii* showing the beginning of the endodermal cavities. (After Chun.)

end, parenchyma-like endoderm; *r*, rib; *stom*, stomodaeum which forms the so-called "stomach."

At this stage the embryo consists of fifty-six cells; eight macromeres, and forty-eight micromeres. The eight macromeres now divide each into two cells of equal size, so as to form a circle of sixteen large cells, and no further divisions take place in them until the micromeres have completed their multiplication.

In subsequent divisions of the micromeres, in the outer octants, the smaller cells divide first and then the larger micromeres divide. In the middle octants also the four smallest micromeres divide first, whilst the eight larger micromeres flatten out and commence to spread over the macromeres, after which they also divide. From this time on, division of the micromeres occurs rapidly, and the sheet of cells to which they give rise spreads more and more over the surface of the macromeres. The macromeres are originally exposed both above and below but the upper opening is narrowed as the edge of the ring of smallest micromeres extends inwards, whilst the larger micromeres rapidly extend downwards over the surface of the macromeres. This over-spreading of the macromeres by smaller cells is termed **epibole.** It is a process often met with in the development of animals and is to be regarded as a variety of **gastrulation.**

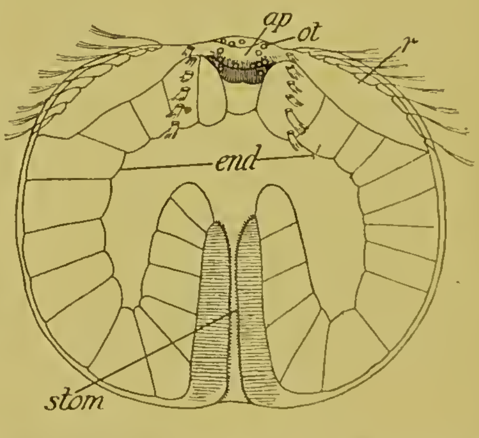

FIG. 74.—Optical section of embryo of *Beröe forskalii* in a later stage of development, with a hollow endodermal sac. (After Chun.)

Letters as before. In addition, *ap*, apical nervous plate; *ot*, otolithic concretions.

There can now be seen, radiating from the upper pole, four streaks of specially small and rapidly dividing cells; these are the forerunners of the "**ribs**" of the adult which carry the comb-like plates of cilia. Each streak corresponds to a pair of ribs.

Just before the lower pole of the macromeres is completely covered by the advancing ectoderm, each macromere buds off downwards, a small cell. We thus get a circle of sixteen small cells at the vegetative pole; these are the rudiment of the so-called **mesoderm**. The macromeres now rotate in such a way that the "mesodermal" cells are rotated upwards and inwards, and eventually come to lie at the upper pole of the macromeres. Here they undergo rapid division and form a cross, the two longer arms of which extend in the direction of the long axis of the embryo, while the two shorter ones are transverse to that axis (Fig. 72).

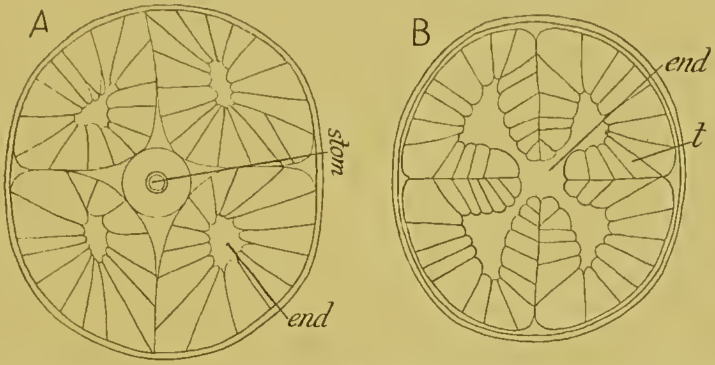

FIG. 75.—Two optical sections through the embryo of *Beröe forskalii*. (After Chun.)

A, through the stomodaeum. B, above the stomodaeum near the aboral pole. *end*, cavity of the gastric sac. *t*, taeniola.

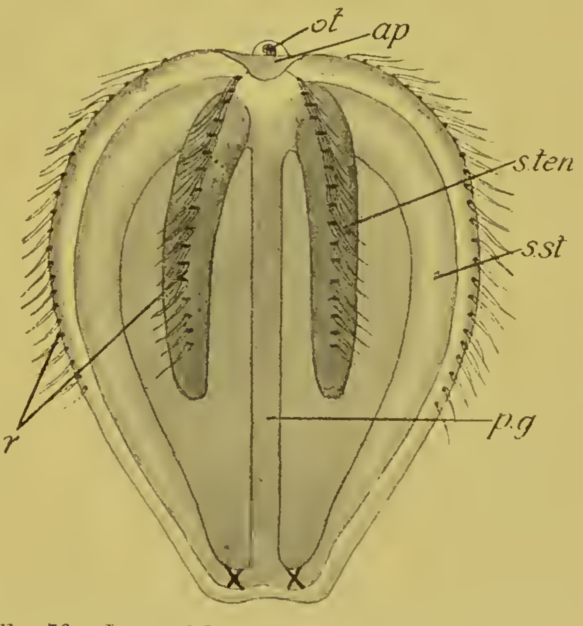

FIG. 76.—Larva of *Beröe forskalii* four days old, viewed from "stomach-plane." (After Chun.)

The paragastric canals have forked and united at × with the sub-ventral or sub-"stomachic" canals. The sub-tentacular canals are still short. Letters as before. ×, forks of paragastric canal; *p.g*, paragastric canal; *s.st*, sub-stomachic canal; *s.ten*, sub-tentacular canal.

The ectodermal skin is now completed, and the macromeres now undergo rapid division, forming a parenchyma-like tissue. Between ectoderm and endoderm jelly appears as a secretion; and into this jelly wandering cells are budded from the cross of "mesoderm" cells. These cells become connected with one another, with the ectoderm and with the endoderm by their processes, so as to produce a cell-network which simulates connective tissue; it is, however, certain that many, if not most, of the cell-processes are contractile, and therefore these wandering cells are to be regarded as muscular.

In the endoderm there appear slit-like spaces between the cells here and there, and these now coalesce so as to form a single roomy, gastric cavity. At the lower pole the **stomodaeum** now makes its appearance as an invagination of the ectoderm. This, as it grows upwards, indents the endodermic sac and constricts it into right and left halves. An indentation occurs in this sac at each side of a line which is at right angles to the plane of the stomodaeum, and in this way a four-lobed sac is formed. We might with perfect justice describe the process as a formation of four ridges or **taeniolae**, which grow into and indent the cavity of the endoderm sac.

Meanwhile, the "**combs**" of cilia have appeared in the regions, where, at an earlier stage, the streaks of rapidly-dividing ectoderm cells were noticeable. Thus the ribs of the adult are established. At the upper pole, groups of stiff cilia supporting otolithic masses are formed, and in this way the **apical sense-organ** is completed.

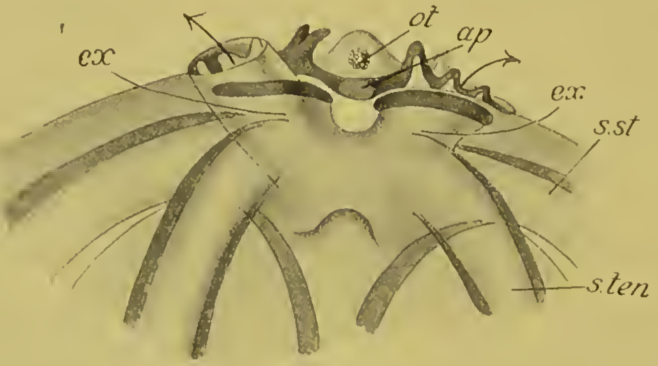

FIG. 77.—Part of apical region of larva of *Beröe forskalii* viewed from stomach-plane. (After Chun.)

Letters as before. *ex*, excretory vessel rising from the funnel.

We have now an oval embryo containing a four-lobed gastric sac, whose walls are formed of vacuolated cells with stiff membranes, and having on one side a mouth leading into a flattened stomodaeum which projects inwards into its interior. Running like the meridians on a globe are eight ribs of thickened ectoderm, each bearing a series of combs of cilia, and converging to a polar plate at the pole opposite the mouth: this plate carries groups of stiff converging cilia which support an otolith where they meet.

At this stage the embryo escapes from the egg-membrane and begins life as a free-swimming larva. The later history has been followed only by Chun and has been deduced from the examination of specimens caught by the Plankton net. From these he concludes that the **meridional canals** appear on the periphery of the endodermic pouches as everted grooves. First the **sub-tentacular** and then the **sub-stomachic** canals appear, then the two **paragastric** canals arise as independent evaginations. All grow down towards the edge of the mouth and here each one forks, and adjacent forks unite with one another, and so a circular canal is formed. This is a peculiarity of *Beröe* as opposed to other Ctenophora. From the gastric sac two canals extend upwards towards the aboral pole of the larva and fuse with the ectoderm at the sides of the apical plate. Here openings are made to the exterior. These canals are called **excretory canals,** and out of them a current of water flows (Fig. 77). It is probable that this current forms the exhalent portion of a respiratory current, and that water enters by the mouth to replace it. Finally, the meridional canals give off short outgrowths which, in one species, anastomose with one another so as to form a network.

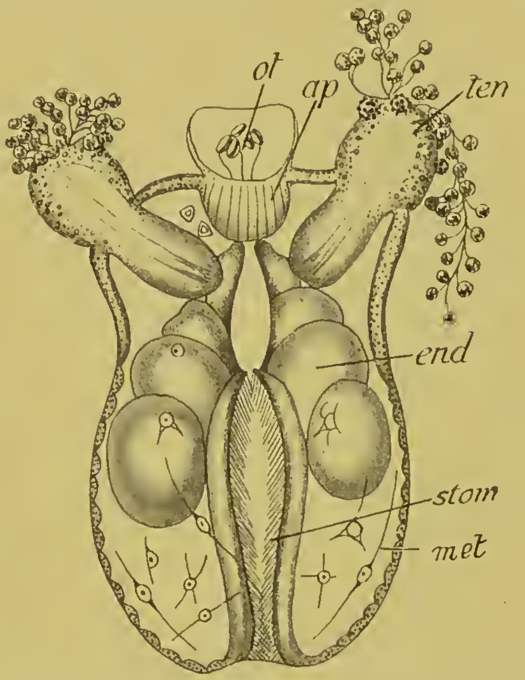

Fig. 78.—The free-swimming larva of *Callianira bialata* viewed from the stomach-plane. (After Metschnikoff.)

ten, freely projecting tentacle; *met*, wandering cells with contractile processes.

CALLIANIRA

The main difference between the development of *Beröe* and that of other Ctenophora lies in the presence of tentacles more or less modified in the other groups.

The best account of the development is that given by Metschnikoff of *Callianira* (1885). From this account we learn that the two main arms of the "mesodermal cross," after having given off a certain number of wandering cells, give rise to the tissue which forms the axis of the tentacles. The **tentacles** themselves appear as invaginations—the **tentacle pockets**—from the base of which the real tentacle sprouts. These tentacle pockets indent the endodermic sac in a plane at right angles to that in which it is indented by the stomodaeum.

AFFINITIES OF CTENOPHORA WITH OTHER COELENTERATA

Now, when we review the account which has just been given of the development of *Beröe*, there is really only one point where any marked similarity with the developmental stages of other Coelenterata shows itself, and this is when the ectoderm has completely invested the macromeres, and these latter have broken up into a mass of cells like plant "parenchyma," with only slits between them. *This stage* *recalls the solid planula stage of other Coelenterates*, the slit-like spaces being comparable to the incipient absorption spaces which are the first stage in the formation of the gastric cavity in other Coelenterates.

Perhaps we might go farther and find in the stage which succeeds to this a similarity to Actinozoon development. In the development of Actinozoa an ectodermal stomodaeum is also formed, and the primary lobing of the enterocoelic sac by four folds in *Beröe* might be compared to the outgrowth of taeniolae or septa in Actinozoa. There would, however, remain an irreconcilable difference, viz., that the first tentacles in Actinozoa sprout from the pouches, whereas in Ctenophora they occur between them—for in Ctenophora, as we have seen, the tentacle pockets cause the formation of taeniolae and therefore alternate with the pockets of the gut.

Reverting, however, to the planula stage common to all Coelenterates, a comparison of the later history of this stage in the various groups reveals the real relationship of the Ctenophora to the rest. In Hydrozoa, Scyphozoa, and Actinozoa, the planula, after a brief period of swimming, fixes itself by its aboral pole, which becomes the root of the future colony; but in Ctenophora the planula never fixes itself but remains free-swimming and develops a complicated sense-organ at the aboral pole.

Hence, if all Coelenterata have sprung from a planula-like ancestor, the Ctenophora must represent a branch which never deserted the free-swimming life, and which in consequence must represent the main stem of Coelenterates, while the other groups, though far more abundant at the present day, must represent degenerate offshoots of this stock. Of these we may suppose that the Actinozoa represent a group which assumed a bottom life later than the rest, and in which, consequently, evolution had gone farther, and a stomodaeum had been formed. In accordance with this conclusion we find that Ctenophora present resemblances to the larvae of the higher forms in far greater degree than do other Coelenterata, for it is to be expected that higher forms would arise from a dominant free-swimming group rather than from a degenerate sessile one.

EXPERIMENTAL EMBRYOLOGY OF COELENTERATA

But though in their later larval life the Ctenophora retain many primitive features, in their earlier embryonic life they have undergone great specialization. This will be made clear by contrasting the results obtained by Zoja (1895–1896), who experimented with the eggs of Hydromedusae, and those obtained by Driesch (1895) and Fischel (1897–1898), who experimented with the eggs of *Beröe*.

Zoja worked with the genera *Liriope*, *Geryonia*, *Mitrocoma*, *Clytia*, and *Laodice*, and he separated the first blastomeres of the segmenting egg with a needle. In the case of *Clytia* and *Laodice* he found that a single blastomere of the 16-cell stage was capable of developing into a normal planula, and that in all cases one of the first four blastomeres could do so. From such a blastomere in *Clytia* a hydroid was reared, and in *Liriope* a fully-developed medusa with four tentacles was reared.

Now Driesch and Morgan (1895) made a number of experiments on the eggs of *Beröe* of which the most interesting are these. By means of a fine scalpel *they cut pieces from the unsegmented egg.* Such mutilated eggs mostly died, but about sixteen out of five hundred survived, and eight of these developed into larvae with a diminished number both of ribs and endôdermal pouches.

From this experiment Driesch and Morgan draw the conclusion that the material required to form definite regions of the embryo is localized in definite parts of the egg. This conclusion is amply confirmed by the result attained from separation of the first blastomeres of the egg by means of a scalpel. Each of the first two blastomeres produces an embryo with four ribs. Each one of the first four blastomeres forms a larva with two ribs only; and further, when one blastomere is separated from the first four the remaining three blastomeres form an embryo with six ribs.

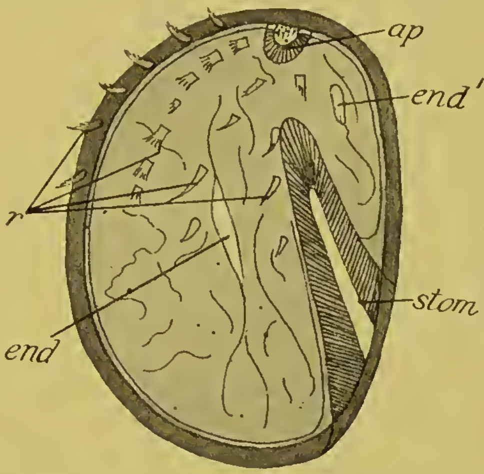

Fig. 79.—An embryo of *Beröe ovata* with four ribs and two endodermal pouches, and a small extra third pouch; obtained by isolating one of the first two blastomeres of *Beröe ovata*. (After Fischel.)

end, one of the two normal endodermic pouches; *end'*, the small extra endodermic pouch; *r*, the four ribs. The other letters as before.

Fischel (1897–1898) improved on Driesch and Morgan's methods: he separated the blastomeres from one another by subjecting the egg to pressure, and by pinching the egg-membrane with forceps. By this means he found he could make one embryo produce three smaller embryos with a lessened number of ribs, and he found that the united number of their ribs amounted to the total of a normal embryo. By pressure he also separated the smallest micromeres, *i.e.* those which give rise to the apical plate, into two portions, and from these resulted an embryo with two apical plates. When pressure was applied in later stages the result was, not to produce several larvae with a lessened number of ribs, but to break up the ribs already formed into several pieces. In larvae with four ribs, produced from one of the first two blastomeres, three endodermal pouches are formed, not two as one would expect (Fig. 79). But Fischel points out that whereas the first two are produced by the ingrowth of taeniolae in the tentacle plane, the small third one owes its origin to the fact that the stomodaeum originates close to one side of the larva, not at the absolute edge as it should do were the embryo a perfect half; in consequence of this fact the growing stomodaeum indents the endodermic sac unevenly, cutting off a little third pouch.

From the fact also that in this half larva, on the side next where the missing half should be, the endoderm is not naked but covered with ectoderm; and from the fact that by separating the lower ends of the macromeres he succeeded in producing a form with two stomodaea, Fischel concludes that although the material destined to form the specialized ribs and the apical plate is originally rigidly localized in the germ, this is not the case with the rest of the ectoderm. This unspecialized ectoderm spreads till it covers all the exposed macromeres, whilst the stomodaeum is formed owing to the action on this general ectoderm of a stimulus proceeding from the lower ends of the macromeres.

From a review of the work of all these experimenters we draw the conclusion that in the eggs of Hydromedusae, up to the 16-cell stage, the part resembles the whole in its constitution; and the question whether one portion shall form only part of an embryo or whether it shall form a whole embryo, depends on whether or not it remains in connection with its fellows. In Ctenophora, however, we must conclude that the part is quite different from the whole, and that the parts destined to form the ribs and apical plate are mapped out even in the unsegmented germ; these parts must, therefore, be represented by portions of the cytoplasm of the egg, not by nuclei.

The egg is incapable of regenerating a lost part, but in this respect a remarkable observation of Chun's (1880) must be borne in mind. He experimented with the eggs of *Bolina* and obtained half larvae just as did Driesch and Fischel with *Beröe.* But Chun kept his half larvae living for weeks, till they developed generative organs on their meridional canals, and he states that the missing half was post-generated. This observation has been recently confirmed by Mortensen.

That an animal in its young stages should possess no powers of regeneration, but should acquire them when it is older, is a curious fact but not unprecedented, for the same thing occurs in Ascidians. We have seen that the specialization of the egg for the formation of organs is a specialization of plasma, but it will be shown later in this volume that this specialization must be regarded as due to an influence emanating from the nucleus of the ovum during the period of its growth and ripening. We must, I think, attribute the secondarily acquired power of regeneration to a reorganization of the cytoplasm, due to renewed influences emanating from the nucleus.

ANCESTRAL MEANING OF THE PLANULA

We have now to consider the meaning of the development of the planula. We have already put forward the hypothesis that the planula represents a free-swimming ancestor common to all

Coelenterata. Now the planula appears under two forms: in Scyphozoa and in some Actinozoa as a hollow two-layered vesicle with a terminal opening, and in Hydrozoa and many Actinozoa as a solid mass of endoderm surrounded by a skin of ectoderm.

Which of these two is the more primitive and which the derived form? In answer to this question we say at once, *the first of these is the more primitive,* because it alone exhibits a structure which is a physiological possibility for a self-supporting animal. A solid internal mass of cells would be quite functionless in an animal that had to get its own living.

The stage of the planula in development is preceded by the stage of the hollow blastula, in all cases which have been thoroughly examined. The hollow blastula is changed into the planula either (*a*) by intucking or invagination of one end, (*b*) by active proliferation of cells proceeding from one end and filling up the interior, or (*c*) by proliferation of cells from the whole internal surface. We have already pointed out that, whereas (*c*) is described only in cases of eggs developing rapidly inside the bell of a vestigial medusa, or with a shortened development in which the hydroid stage is almost eliminated and the egg develops directly into a medusa, and that the assertion of its occurrence even in these cases may be based on a mistake in the observations; on the other hand (*a*) and (*b*) occur in eggs with a long larval development.

We may take it therefore that these latter methods of endoderm formation represent the least modified form of development, and that the ancestral blastula was developed into the ancestral planula by a proliferation of cells at one pole only, or by an invagination of the cells forming the wall of the blastula at this pole.

If this be admitted, however, we have no difficulty in deciding that invagination must be more primitive than polar proliferation. Our reason for that decision is that polar proliferation would be meaningless in an adult animal, whereas invagination means, primarily an increase in surface area of a portion of the animal, and secondarily an inbending and the consequent continuous preservation of a cavity between the invaginated cells, which cavity is destined to contain food.

In fact, as Korschelt and Heider point out, a ciliated animal, progressing forward in one direction, tends to create suction behind it, so that particles struck backwards by the cilia tend to accumulate there; just as may be observed at the tail of an express train as it dashes past a station. Here then would be sufficient inducement for the tendency to increase and exaggerate the function of ingestion —a function which all the cells of the planula originally must have possessed,—and so we may suppose that an endoderm and an ectoderm would become specialized from an indifferent layer of cells.

As the endoderm cells increased in number it became necessary that they should find room, and this they did by bending inwards, and so the planular stage is reached. When this stage is repeated in ontogeny, and when the food necessary for the embryo is stored up within its cells in the form of yolk-grains, and has not to be sought for outside, then the inbending can be replaced by solid proliferation because the larval gut is no longer a functional organ.

We are thus led to form the following conception of the past history of the lower Metazoa. A widespread and dominant race of blastula-like animals once swarmed in the primeval seas. Some of these took a creeping life and eventually gave rise to the group of sponges; others kept to the free-swimming life and developed into planulae, and so gave rise to the Coelenterata. Some of these planulae, by the specialization of the cilia into comb-like locomotor organs, became Ctenophora; whilst the remainder adopted a fixed life and attached themselves by their aboral poles. This change occurred in different divisions of the stock at different stages of the evolution of the internal organs of the planula ancestor, and in this way the groups of Hydrozoa, Scyphozoa, and Actinozoa arose.

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CHAPTER V

PLATYHELMINTHES

Classification adopted—

- **Platyhelminthes**
  - **Turbellaria**
    - **Dendrocoela**
      - **Polyclada**
      - **Triclada**
    - **Rhabdocoela**
      - **Acoelida**
      - **Rhabdocoelida**
      - **Alloiocoelida**
  - **Trematoda**
  - **Cestoda**

THE phylum of Platyhelminthes or Flat-worms agrees in one point of structure of great importance with the Coelenterata. The alimentary canal may, it is true, entirely disappear in some members of the phylum which are internal parasites and which live by the absorption of fluids through the skin, but when it is present it has only a single opening, **the mouth,** which serves both for ingestion and egestion. Further, between alimentary canal and skin there is no real body-cavity, the space being occupied by a ground substance comparable to the jelly of a Coelenterata. This ground substance is invaded by numerous stellate cells connected together by their processes, some of which become muscular and contractile; in these two respects this tissue resembles especially the jelly of a Ctenophore.

When we survey the development of the eggs of this group, we find a feature common to a large number which entails modifications of the early life-history of a far-reaching character, and renders observation correspondingly difficult. This peculiarity is that the eggs are enclosed in large numbers in a common capsule, and that of this number only one is destined to develop into an embryo, whereas the rest, termed "**vitelligenous,**" *i.e.* yolk-bearing cells, are destined to become its food.

Now, as a result of this arrangement, the earlier stages of development are modified out of all recognition. The first-formed blastomeres are stated to separate from each other completely, then to wander to the periphery of the vitelligenous cells, which they surround, and then to join together to form an embryo. In fact one could not have a better example of the disturbing influence of a superabundance of food, which we have already seen reason to believe is one main cause of variation.

Now it is obvious that these peculiarities are secondarily derived, that they are not primary characteristics; yet they range throughout the entire groups of the Trematoda and Cestoda, and in the group of the Turbellaria they are found in Triclada amongst Dendrocoela, and in Rhabdocoelida and Alloiocoelida amongst Rhabdocoela. In the curiously modified Acoelida, where the gut has no lumen and its cells are indistinguishable from those of the parenchyma, this peculiarity does not occur, nor does it occur in the group Polyclada of the Dendrocoela.

Now the Acoelida support life, as Keeble (1907) has shown, by means of their association with a plant; this accounts for the absence of a hollow alimentary canal, and it cannot therefore be regarded as a primitive feature. The Polyclada, on the other hand, retain the power of swimming by cilia, and they exhibit other features of a very primitive character; it is to their ontogeny, therefore, that we naturally turn for light on the origin of the group.

The pioneer in this work has been Lang (1889), who has worked at the development of several European genera. We nevertheless take for type the American form *Planocera*, which has been worked out with great care by Surface (1907), because it is the most recent work and the most modern methods have been employed for it, whereas Lang's work dates back twenty to thirty years. Nevertheless the outcome of Surface's work is to support Lang's main conclusions, and to suggest strongly that the eggs of all the genera of Polyclada segment very much in the same way, so that what is here recorded of *Planocera* will be found to be nearly correct for the European species also.

PLANOCERA

Planocera lives in the mantle-cavity of the Gastropod *Sycotypus*. It does not feed—so far as is known—on the tissues of its host, but uses its host's mantle-cavity as a convenient retreat. It is therefore a commensal, not a parasite. The adults were obtained by opening the branchial cavities of specimens of the Gastropod. They were kept in vessels containing clean sea-water through which a current of air was allowed to bubble. They laid their eggs in these vessels, and the eggs lived till the free-swimming larva escaped.

The eggs of *Planocera* are minute, being only ·1 mm. in diameter, and they are laid in capsules, usually only one in each capsule, and the capsules are embedded in a gelatinous slime. Occasionally two eggs are laid in a capsule, and then both become normal embryos. No attempt was made by Surface to extract the eggs from the capsules, but they were examined living and were then preserved in fluids of penetrating power which reached and preserved them whilst still in the capsules.

The preserving fluids employed with most success were a mixture of 3 parts corrosive sublimate and 1 part acetic acid dissolved in 95 per cent alcohol; and Gilson's fluid—a mixture of corrosive sublimate and nitric acid. Other fluids were tried but did not give good results. The preserved eggs were stained in Grenacher's haematoxylin, and cleared in xylol and mounted in Canada balsam. The operation of the clearing agent was facilitated by piercing the capsule with needles.

The embryo develops within the capsule until the sixth day, when it escapes as a free-swimming larva of a peculiar type known as **Müller's larva.** This larva swims about at the surface for some time, but eventually sinks to the bottom and then takes on the form and habits of a Polyclade.

In the development of *Planocera* we meet for the first time with what is termed "**spiral cleavage**" of the egg. This type of segmentation of the egg into blastomeres is very widely distributed. It is found in all Polyclada which have been studied, it is universal amongst Annelida, and it is found in all Mollusca except Cephalopoda. In the Nemertine worms it also appears, though in a very primitive form. Developments of this type have been studied in great detail by zoologists of the American school, and they have invented a nomenclature which is applicable to all such developments; we shall endeavour to make this clear, and shall adopt it in the description which we give. The object aimed at in these studies is to trace back definite organs of the embryo or larva to individual blastomeres of the egg in its early stages of cleavage. This is termed tracing the **Cell-lineage** of the organs.

The general features of "spiral cleavage" are these. The egg divides as usual into two and then into four cells, by two cleavage planes at right angles to one another. These four cells then divide into eight, which lie in two tiers of four, one above the other. So far there is nothing peculiar about the type. But the four cells nearest the animal pole of the egg are usually much smaller than the others, and are termed **micromeres,** whilst their larger sisters are termed **macromeres.** Further, the spindles by which the nuclei of the micromeres are separated from those of the macromeres are not vertical but oblique, with the result that the micromeres are situated opposite the furrows between the macromeres, and, so to speak, alternate with them.

In Planocera, and in the great majority of cases of spiral cleavage, in the transition from the 4- to the 8-cell stage, each spindle is so inclined that the micromere lies at the upper right-hand corner of the macromere from which it has separated (compare 1A and 1*a* in Fig. 80); that is, the upper right-hand corner when viewing the egg from above the animal pole. Such a direction of cleavage is termed **dexiotropic.** In only a few cases, in which reversed cleavage, as it is called, occurs, the spindles preparatory to the formation of the 8-cell stage are so directed that the micromere lies at the left-hand upper corner of the macromere. Such cleavages are termed **laeotropic.**

The 8-cell stage is succeeded by a 16-cell stage. The micromeres divide into upper and lower cells by laeotropic spindles, and by four laeotropic spindles four new micromeres are budded off which alternate with the lower daughter cells of the first micromeres. (Fig. 81.)

The 16-cell stage is succeeded by a 32-cell stage. By dexiotropic spindles the macromeres bud off a third set of micromeres. The second micromeres divide into upper and lower cells, whilst each daughter of the first set of micromeres divides into an upper and lower cell.

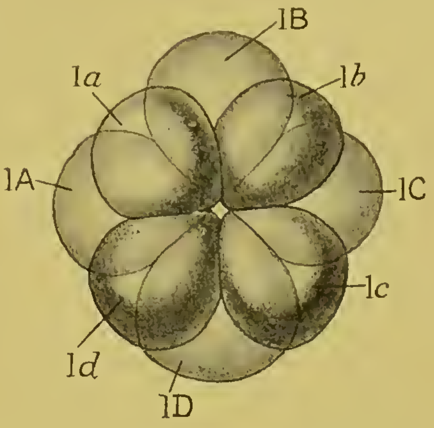

Fig. 80.—Developing egg of *Planocera inquilina.* Eight-cell stage viewed from animal pole. (After Surface.)

The subsequent history of the embryo proves that in *Planocera*, as in every Annelid and Mollusc that has been examined, the three groups of micromeres and their daughters constitute the entire ectoderm, whilst what is left of the macromeres, after the separation of these micromeres, gives rise to the endoderm and mesoderm.

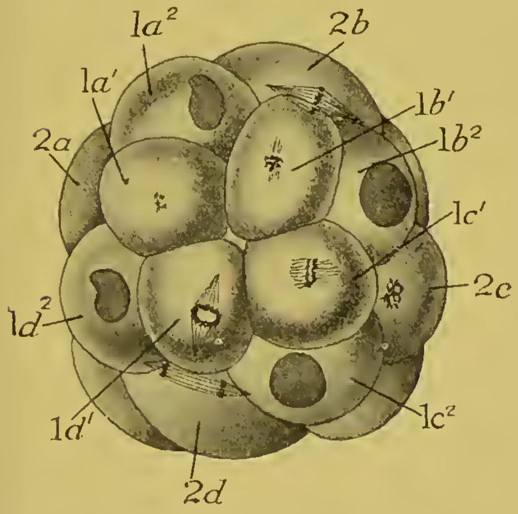

Fig. 81.—Developing egg of *Planocera inquilina.* Sixteen-cell stage viewed from the animal pole. (After Surface.)

Now if the four macromeres were precisely equal in size it would be of course impossible to distinguish them from one another, but in *Planocera*, as in the vast majority of cases, one is slightly larger than the rest and distinguishes itself by peculiarities in its development after the micromeres have been given off; this macromere is denominated D. In all cases where it is recognizable from the first it forms a landmark by means of which the cleavage planes of the egg can be correlated with the planes of symmetry of the adult.

It is thus found that D is situated on what will be the posterior side of the embryo in the middle line. The other cells are named A, B, and C, following each other round a circle from left to right, in the same direction as the hands of a clock move when viewed from above. A and C are situated on the left and right sides respectively whilst B is median and anterior. B and D (in *Planocera*) are larger than A and C and meet each other in short contact plane at the vegetative pole, whilst A and C meet each other in a short contact plane at right angles to this at the upper or animal pole of the egg.

Now the division of these four cells used to be described so that A was said to bud off a micromere *a*; B a micromere *b*; and so on. But this is not a logical description of the event, for the so-called budding off of *a* is really the division of A into two cells, a larger and a smaller, and neither should bear the same name as belonged to their common mother. So that under an improved nomenclature, when the four first blastomeres divide, the cells to which they give rise are denominated 1*a* and 1A, 1*b* and 1B, 1*c* and 1C, and 1*d* and 1D respectively; the smaller letter in each case denoting the micromere and the larger the macromere (Fig. 80).

When the second group of micromeres is given off, 1A divides into 2*a* and 2A, and so on, but to denote the divisions of the already formed micromeres a different notation is used. Thus 1*a* is said to divide into $1a^1$ and $1a^2$, where ^{1} denotes the daughter nearest the animal pole, and ^{2} the one nearest the vegetative pole of the egg (Fig. 81). When in the 16-cell stage $1a^1$ divides, its daughters are denoted $1a^{11}$ and $1a^{12}$, on the same principles.

It will easily be seen that this notation is capable of indefinite expansion. It has, however, one serious defect. Sometimes the two daughters resulting from the division of a cell lie side by side at the same distance from both poles of the egg. In this case ^{1} is held to denote the right-hand daughter, and ^{2} the left-hand daughter. But this is confusing, because the eye learns to associate the symbol ^{2} with a lower position in the egg than ^{1}, and it is a strain to grasp the fact that the cell $2a^{211}$ may be higher in the egg than $2a^{121}$.

Woltereck in his paper on *Polygordius* (see Chapter VII.) gets over this difficulty by using the letters (*r*) and (*l*) to denote right and left instead of the numbers ^{1} and ^{2}, if the two daughters lie to the right and left of the median plane of the egg. Where, however, they both lie at the side of the egg, then the letters (*a*) and (*p*) for anterior and posterior are employed. This practice completes the perfection of the nomenclature, but it is unfortunately not employed by Surface and the other American writers.

Each group of micromeres is known as a **quartette**, and all the cells resulting from the divisions of one of the first four blastomeres are known collectively as a **quadrant** of the egg.

To return to the special case of *Planocera*. The cell D is distinguishable as the largest of the first four, and, as we have seen, it occupies what afterwards turns out to be the posterior pole of the embryo. When once this is recognized the egg is so placed that D is posterior.

In the formation of the first micromeres D divides first, B follows, and A and C divide simultaneously, so that division takes place in the order of the size of the cell. The first micromeres are nearly as large as the macromeres, but in the European genus *Discocoelis* the inequality in size between micromeres and macromeres is much more marked. In the next period of cell-division the second micromeres are formed by a laeotropic division.

The first quartette divides so as to give rise to two tiers of four cells, all being of nearly equal size. The lower are of course $1a^2$, $1b^2$, $1c^2$, and $1d^2$, and only divide once or twice again.

The third quartette is then formed, and the second quartette divides, each member giving rise to an upper smaller and a lower larger cell ($2a^2$), etc. The cells $1a^1$-$1d^1$ divide into lower, somewhat smaller cells, $1a^{12}$, etc., and upper larger cells $1a^{11}$, etc.[1] The cells $1a^2$-$1d^2$ divide similarly. When the egg in this stage is viewed from its lower or vegetative pole, the cells belonging to the second and third quartettes are seen to alternate with one another and together to form a girdle round the egg just below its equator (Fig. 82).

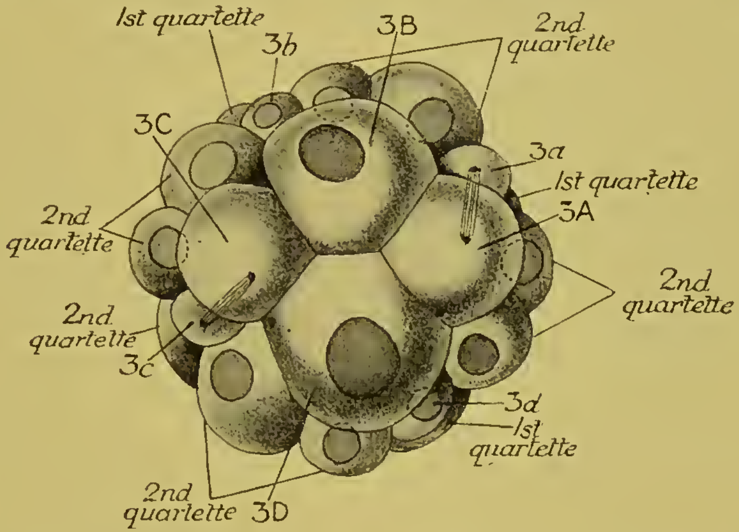

FIG. 82.—Developing egg of *Planocera inquilina*. Thirtytwo-cell stage viewed from vegetative pole. (After Surface.)

After the 32-cell stage, distinct periods of division, affecting the cells of the egg simultaneously, cease, and hence the different groups of cells must be dealt with separately.

An extraordinary feature in *Planocera* is the formation of the fourth quartette. In this case Surface still terms the upper daughter a micromere and the lower a macromere, in order to facilitate comparison with other eggs of the same type of cleavage. But in the formation of this quartette the daughter cells, the so-called "macromeres" (*mac*, Fig. 84), occupying the vegetative pole of the egg, are far smaller than their sister cells, the so-called fourth quartette of micromeres. According to Surface these minute macromeres are destined to undergo ultimate absorption, and take no further part in the development. Their nuclei degenerate, the chromatin in each becoming aggregated into a single deeply-staining mass; in fact these degenerating nuclei form an excellent landmark by which the egg can be orientated.

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[1] The hyphen between the names of two cells indicates that these names are an abbreviation for the whole quartette. Thus $1a^1$-$1d^1$ means $1a^1$, $1b^1$, $1c^1$, $1d^1$; $1a^{11}$, etc. likewise means the series $1a^{11}$, $1b^{11}$, $1c^{11}$, and $1d^{11}$

Of the so-called micromeres of the fourth quartette, which are really the largest cells in the egg, three, viz. 4*a*, 4*b*, and 4*c*, undergo no further development, but form masses of yolk which gradually degenerate and are absorbed by the cells of the gut, when this is formed. The fourth, 4*d*, contains the greater part of the original macromere D, and gives rise to the whole of the endoderm and a considerable portion of the so-called mesoderm, that is, the so-called **parenchyma** or mass of muscular and connective tissue cells which intervenes between ectoderm and endoderm.

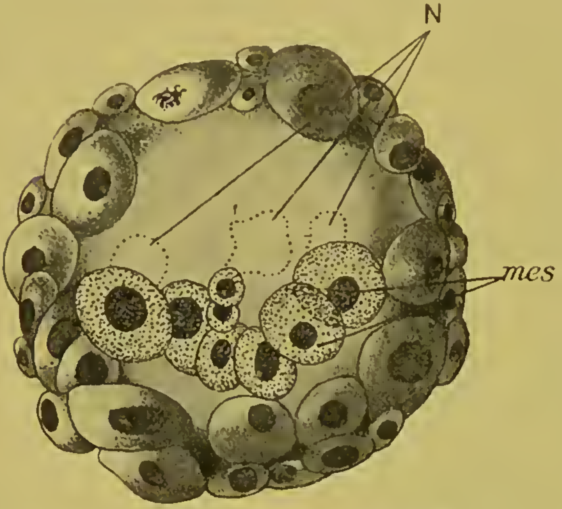

FIG. 83.—Optical section of developing egg of *Planocera inquilina* viewed from posterior pole. (After Surface.)

mes, the so-called mesodermic bands; N, nuclei of the three inactive cells, 4*a*, 4*b*, and 4*c*.

This cell, 4*d*, divides into an inner cell termed by Surface $4d^2$, and an outer, $4d^1$. (This nomenclature is incorrect as $4d^2$ certainly lies above $4d^1$.) $4d^2$ divides into two exactly equal daughters lying right and left of the median plane of the embryo. Just after this the outer cell, $4d^1$, divides in an exactly similar fashion. The inner cells bud off two very small cells with deep-staining nuclei (*end'*, Fig. 84); they lie just above the spot where the pharynx will be formed and are incorporated in the wall of the alimentary canal.

Their larger sisters divide so as to form each a string of cells directed obliquely forwards on either side (*mes*, Figs. 84 and 86). These strings or bands are compared by Surface to the similar bands which are found in Annelidan and Molluscan embryos, which arise from the division of 4*d*, and which give rise to the coelom, but this is doubtful since the cells in question only occasionally take on a band-like form. They give rise, according to Surface, to a large portion of the general parenchyma of the body, but the parenchyma near the lower pole of the embryo is derived from descendants of cells of the second quartette, which migrate inwards and supply muscles for the stomodaeum (*mes.ect*, Figs. 84 and 86); this parenchyma is known as

mesectoderm in order to distinguish it from the parenchyma derived from $4d^2$ which occupies the more dorsal portion of the embryo, and which is termed **mesoderm.**

The endoderm, with the exception of the two small cells budded from $4d^2$, is all derived from the divisions of $4d^1$. This cell, as we have seen, divides into right and left sisters; each then divides several times so that six or eight large cells are formed, lying at the posterior pole of the egg (*end*, Fig. 84). Soon afterwards the spreading edge of the ectoderm, consisting of the daughters of the second and third quartettes of micromeres, reaches the lower pole of the egg and covers this group of endoderm cells.

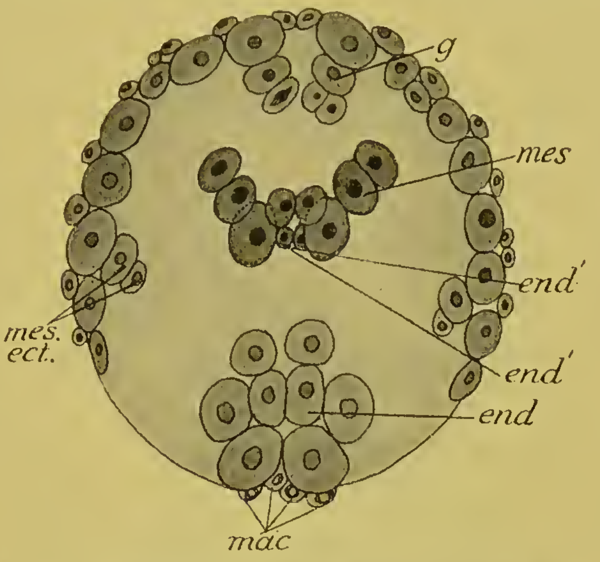

Fig. 84.—Diagrammatic frontal section through egg of *Planocera inquilina* at a later stage of development than that represented in Fig. 83. (After Surface.)

mes, mesodermic bands; *end*, endoderm; *end'*, small endodermal cells formed from mesoderm rudiment; *mac*, vestigial macromeres; *mes.ect*, cells of ectodermal origin which migrate inwards to form muscles of the stomodaeum or larval pharynx; *g*, cells forming rudiment of brain.

During this time the other three micromeres of the fourth quartette have remained undivided, but by the pressure of the daughters of $4d^1$ they are pressed higher up in the egg; their yolk-granules begin to coalesce so as to form fat-like drops, and these drops run together so as to form a limited number of enormous spherules. The nuclei remain large and conspicuous (N, Fig. 83).

When the ectoderm has completely covered the egg, an invagination takes place at the lower pole; this is destined to form the stomodaeum of the larva. The four minute "macromeres" become pushed in before it and ultimately disappear altogether. Then the mass of cells derived from $4d^1$ begins to separate one cell from another, in the middle, so as to give rise to a cavity, the future gut-cavity.

As the endodermic cells multiply they spread in an amoeboid fashion over the outer surfaces of the yolk-spheres derived from $4a$, $4b$, and $4c$, so that these are included within the lumen of the alimentary canal and are absorbed (Fig. 86). But in *Discocoelis*, according to Lang, $4a$ and $4b$ and $4c$ bud off small cells which take part in the formation of the alimentary canal, so that in *Planocera* one member of the quartette does the work which in *Discocoelis* is done by all four members. This kind of variation is not uncommon in eggs with spiral cleavages; it has been observed both in Annelida and Mollusca.

We must now return to a more detailed study of the three ectodermal quartettes. Turning our attention to the first quartette, we left it at the stage where it consisted of sixteen cells, viz. $1a^{11}$-$1d^{11}$, $1a^{12}$-$1d^{12}$, $1a^{21}$-$1d^{21}$, and $1a^{22}$-$1d^{22}$; $1a^{11}$ is rather larger than $1a^{12}$, and $1a^{21}$ is larger than $1a^{22}$. $1a^{11}$-$1d^{11}$ bud off four small cells which occupy the uppermost pole of the egg, these are the so-called **apical cells,** $1a^{111}$-$1d^{111}$; they are little more than nuclei, the cytoplasm being reduced to a thin plate in which cell limits are not discernible (*ap*, Fig. 85).

Now this type of division by which small sisters are separated at the surface whilst larger sisters remain more deeply situated, is repeated afterwards by the larger daughter cells which have resulted from the division just described. These cells, $1a^{112}$-$1d^{112}$, divide each into an upper very small cell, $1a^{1121}$-$1d^{1121}$, and a lower larger cell, $1a^{1122}$-$1d^{1122}$. For the third time a similar division takes place. The lower cells resulting from this last division, $1a^{1122}$-$1d^{1122}$, give rise to four very small cells situated externally, and to four much larger cells situated internally.

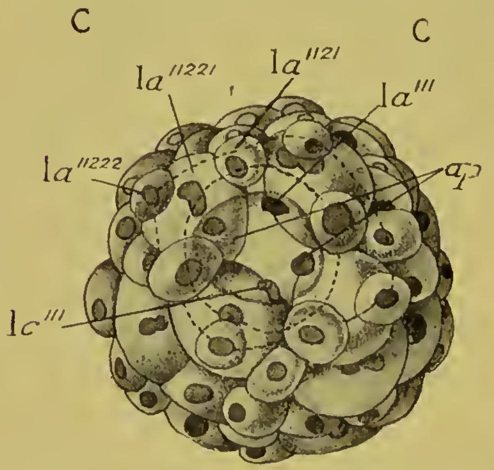

FIG. 85.—Developing egg of *Planocera inquilina* in a late stage of segmentation, viewed from animal pole. (After Surface, slightly altered.)

ap, thin apical plate without distinction of cell outlines formed by the cells a^{111}, b^{111}, c^{111}, and d^{111}. The dotted lines indicate concentric circles of small cells near the animal pole of the egg. A third circle is constituted by the cells composing the apical plate.

The larger daughter cells arising from the last division are denominated $1a^{11221}$-$1d^{11221}$. Once again they undergo a similar division, each giving rise to a large internal cell and a small external one.

Thus we finally get four concentric circles of small cells at the upper pole of the egg, whilst the four larger internal cells resulting from the last division form the rudiment of the **cerebral ganglion** (*g*, Fig. 84). The cells, $1a^{121}$-$1d^{121}$, divide each into two daughter cells of equal size, but the cells, $1a^{21}$-$1d^{21}$ and $1a^{22}$-$1d^{22}$, divide each into a large internal and a small external cell.

Turning now to the second quartette, each member of course divided at the time that the third quartette was given off, for until the 32-cell stage all the cells of the egg divide together. Thus we have eight cells, $2a^{1}$-$2d^{1}$ and $2a^{2}$-$2d^{2}$; if we take the divisions in quadrant as an example of the whole we find that $2a^{1}$, divides into a smaller external cell, $2a^{12}$, and a larger deeper cell, $2a^{11}$, whilst $2a^{2}$ divides into two equal cells one above the other. The lower of these cells lie in the furrows between the large cells of the fourth quartette, and each of them divides again into two equal cells, the lower of which $2a^{222}$, etc. reaches and touches the corresponding macromere, thus completing the covering of the egg with ectoderm. $2a^{11}$, etc. on the other hand, which had already budded off $2a^{12}$ as a smaller external cell, repeats the process, giving rise to $2a^{112}$ externally, and $2a^{111}$ internally. This last cell, $2a^{111}$, with the corresponding cells formed

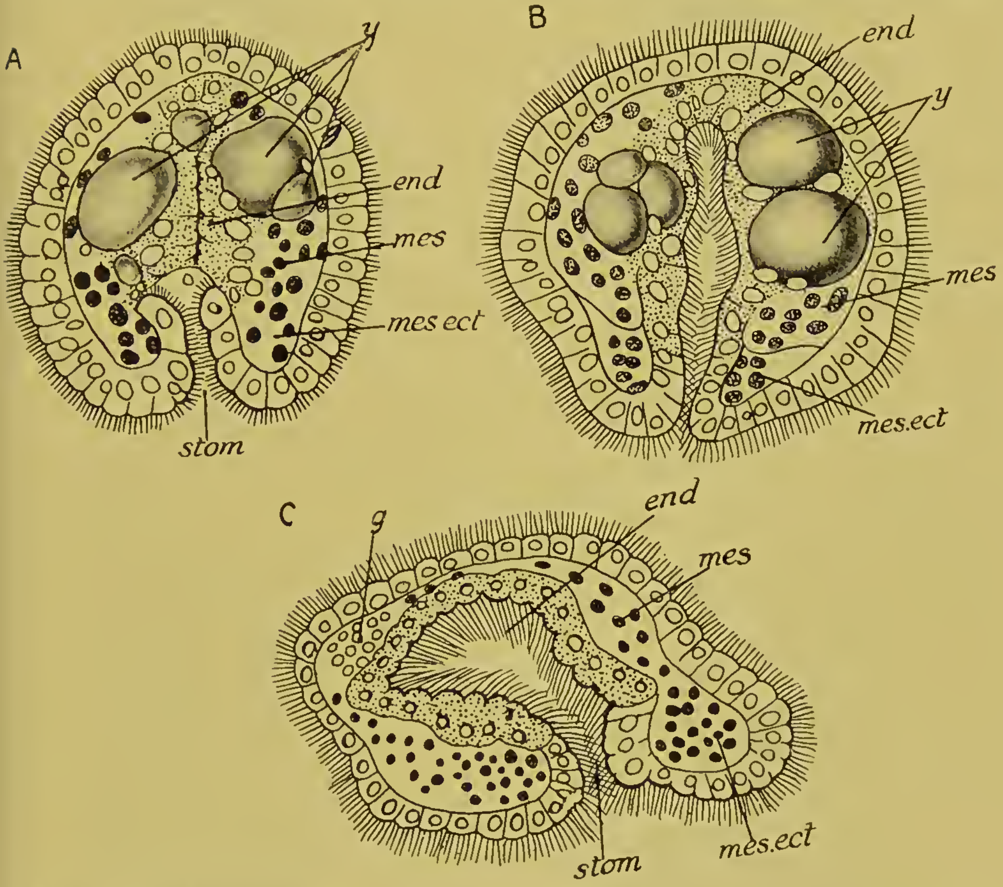

FIG. 86.—Three longitudinal sections through developing embryos of *Planocera inquilina*. (After Surface.)

Letters as in Fig. 84. In addition, *y*, yolk spheres resulting from the disintegration of the cells, $4a$, $4b$, and $4c$; *stom*, stomodaeum. A, the endoderm forms a solid mass of cells in which the gut-cavity is just beginning to appear. B, later stage, the gut-cavity has appeared. C, longitudinal section of Müller's larva just after its escape from the egg-capsule.

by the other members of the quartette $2b^{111}$, $2c^{111}$, $2d^{111}$, give rise to the mesectoderm, lying at the lower pole of the egg, which almost certainly forms the musculature of the stomodaeum which serves as larval pharynx.

The third quartette of micromeres undergoes only equal divisions and all the daughters remain at the surface and are incorporated in the ectoderm. Beyond this stage Surface could not trace the ancestry of the cells of the embryo with any certainty.

We have now traced the divisions of the egg to the formation of an oval embryo, at the lower pole of which is an ectodermal stomodaeum opening above into an irregular endodermic sac lined by small cells, all trace of the yolky cells of a former stage having disappeared whilst the upper pole of the egg is occupied by a plate of extremely small cells, beneath which are ganglion cells.

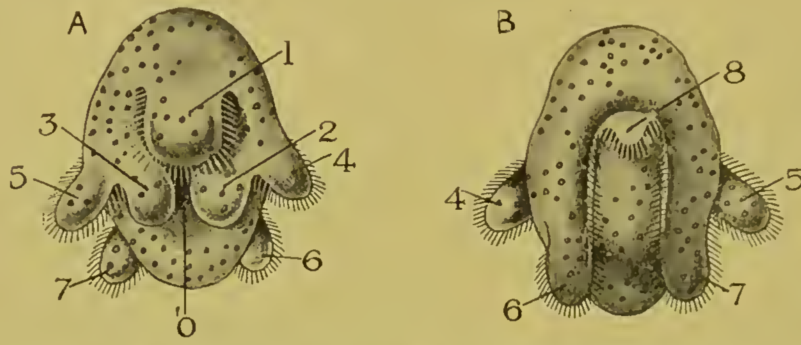

FIG. 87.—A, dorsal, and B, ventral views of the free-swimming larva of *Yungia*. (After Lang.) 1-8, ciliated lobes; *o*, mouth.

Ciliated lobes now appear just below the equator; there are eight of these, and from their position they must be formed by the descendants of $1a^2$-$1d^2$. The larva, which has rotated in the capsule by the action of these cilia, now escapes and leads a free-swimming life. It gradually undergoes changes which have not been followed by Surface in detail, but which Lang has described in *Yungia*.

METAMORPHOSIS OF THE LARVA OF *YUNGIA*

Already at the time of its escape from the egg capsule the larva has lost its primordial radial symmetry. The mouth, which originally occupied the lower pole of the embryo, has become shifted on to one side, which afterwards turns out to be the ventral side, owing to inequality in the rate of growth of the two sides of the embryo (Fig. 86, C). Of the eight ciliated lobes which have been formed, one overhangs the mouth, one is mid-dorsal, and six are lateral, forming three pairs, the corresponding members of which are situated to the right and left of the middle line (Figs. 87, 88, 89). The band of cilia which fringes these lobes is continuous from one lobe to another, so that it forms a continuous girdle surrounding the larva. As the larva grows older it becomes longer and flatter; eye-spots (*oc.* Fig.

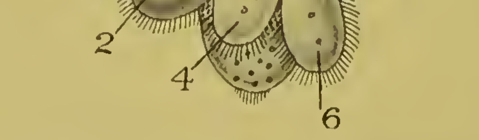

FIG. 88.—Lateral view of the free-swimming larva of *Yungia*. (After Lang.)

89 B) appear over the region occupied by the apical cells of the embryo, and the ciliated lobes become less and less prominent

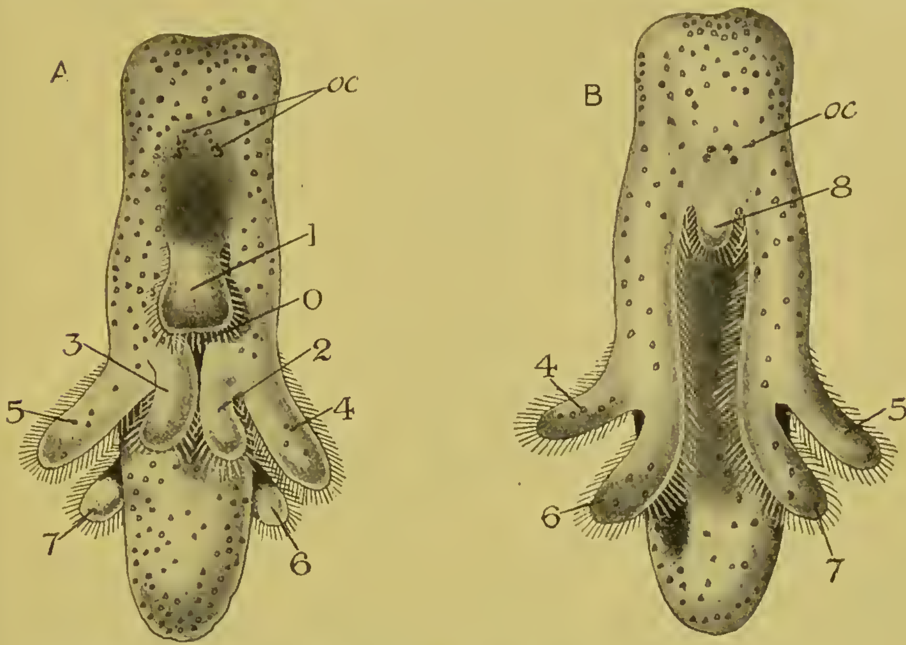

FIG. 89.—A, dorsal, and B, ventral views of larva of *Yungia aurantiaca* in which metamorphosis is beginning. (After Lang.)

oc, eyespots.

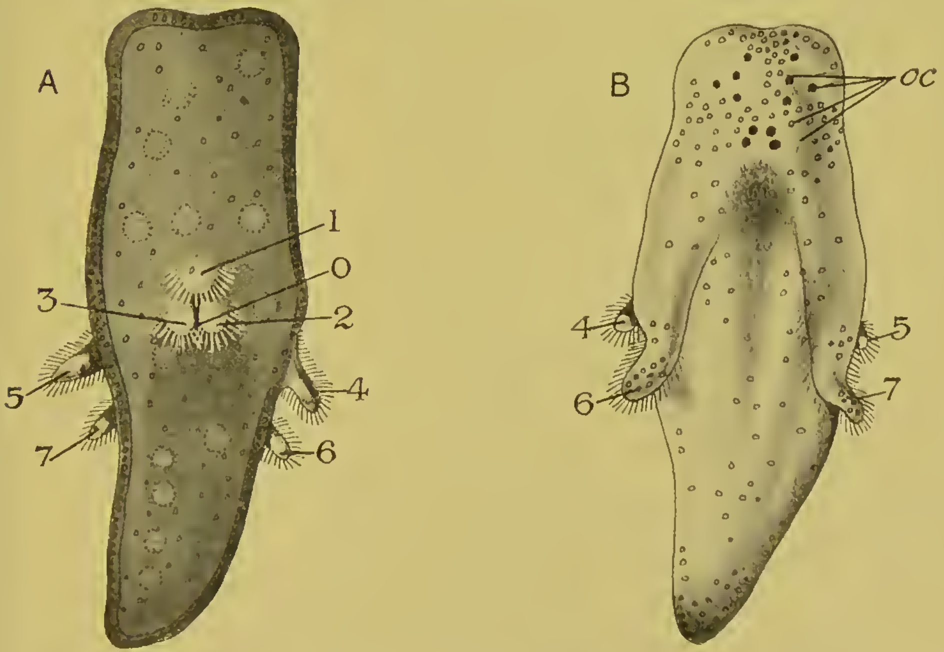

FIG. 90.—A, dorsal, and B, ventral views of larva of *Yungia aurantiaca* in which metamorphosis is almost complete.

(Fig. 90) and finally disappear altogether, so that the larva gradually becomes transformed into a Polyclade worm.

Of the internal changes which meanwhile take place the most noteworthy is the formation of the adult pharynx. This arises as a ring-shaped evagination of the innermost part of the larval stomodaeum (*stom*, Fig. 91), lined by thin cells, from the bottom of which the pharynx arises as a ridge-like thickening (*ph*, Fig. 91). During larval life this evagination is virtually a closed cavity which communicates with the stomodaeum by the narrowest slit. As the adult condition is attained, the outer part of the larval stomodaeum is everted and its walls become parts of the external surface of the body, the evagination alluded to above opens widely into the inner portion of the stomodaeum and becomes the adult pharyngeal sheath surrounding the pharynx (Fig. 92). During larval life muscles become differentiated from cells of the parenchyma, and some of these (*l.musc*, Fig. 91) which serve to elevate and depress the ciliated processes are of a provisional character, whereas others (*d.musc*, Fig. 91) persist as the dorso-ventral muscles of the adult. The endodermic sac develops an anterior pouch

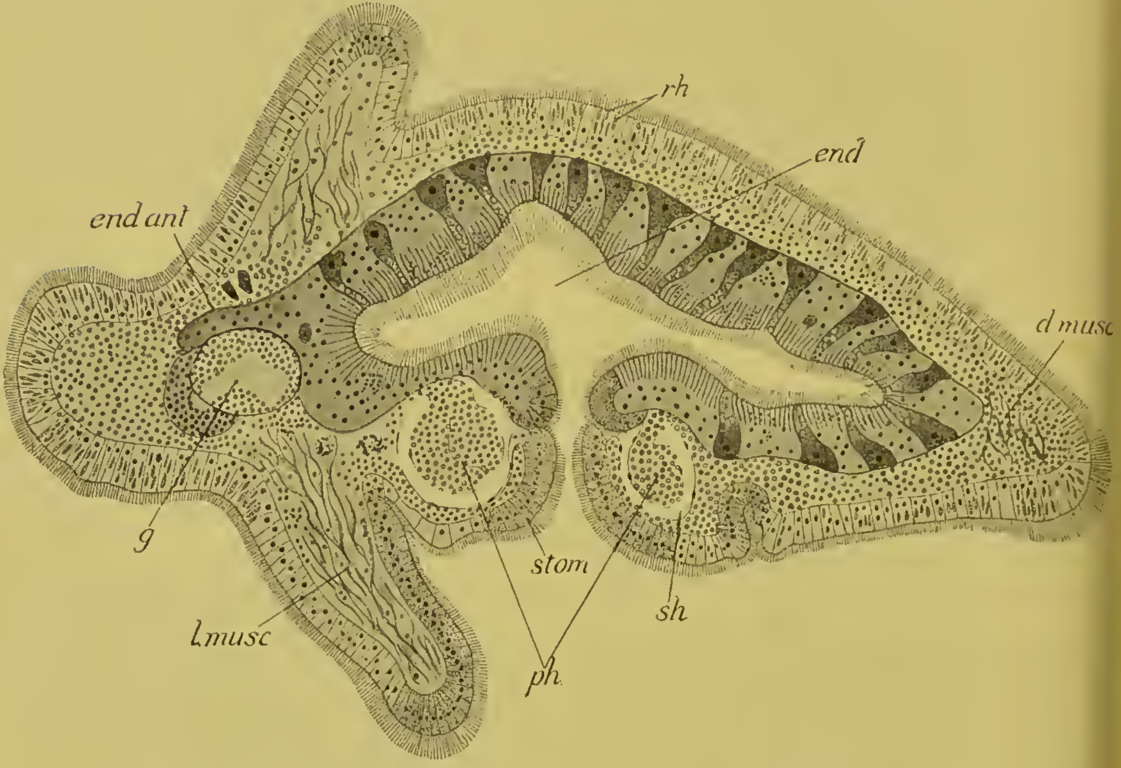

FIG. 91.—Median sagittal longitudinal section through larva of *Yungia aurantiaca*. (After Lang.)

end, endodermic sac; *end.ant*, anterior diverticulum of gut passing over brain; *d.musc*, dorso-ventral muscles; *g*, brain; *l.musc*, larval muscles; *ph*, rudiment of adult pharynx; *sh*, pharynx-sheath; *stom*, stomodaeum or larval pharynx; *rh*, rhabdites in ectoderm-cells.

which projects forwards over the brain (*end.ant*, Figs. 91 and 92), and which has been compared by Lang to the so-called excretory canal of a Ctenophore (*v. infra*). The origin of the genital organs was not observed by Lang. The characteristic **rhabdites** appear in the ectoderm cells (*rh*, Fig. 91) during larval life.

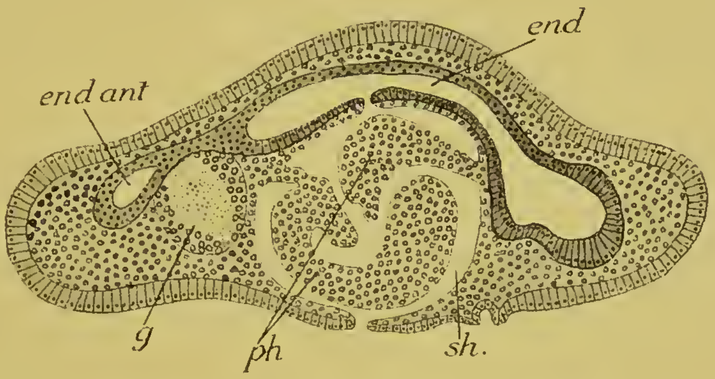

FIG. 92.—Median sagittal section through a young Polyclade worm (*Yungia aurantiaca*) just after its metamorphosis. (After Lang.)

Letters as in preceding figure.

THE EVOLUTION OF PLATYHELMINTHES

Now Lang (1889) was led by the study of the European genera to see a strong resemblance between this type of development and that of a Ctenophore, and he therefore suggested that Polyclada, and, inferentially, all Platyhelminthes, are merely Ctenophora which have become adapted to a creeping form of life.

In Polyclada as in Ctenophora there are large macromeres which bud off smaller micromeres, and from these last the ectoderm is formed. In both groups there is an ectodermal stomodaeum occupying the lower pole of the embryo, and at the upper pole we find the main nervous centre. Further, in both, the primary locomotor organ consists of eight ciliated ridges of ectoderm, and Lang has shown that in Müller's larva the cilia on the ciliated processes are joined edgewise so as to form combs. It would perhaps be better to compare the ascending loops of the ciliated band which intervenes between two of the larval processes, to the rib of a Ctenophore. Müller's larva would, on this hypothesis, represent the pelagic Ctenophore-like ancestor of Polyclada.

This hypothesis is by far the most plausible that has yet been put forward to account for the extraordinary Platyhelminthes, and it is strengthened by the reflection that a creeping Ctenophore, *Ctenoplana*, exists, in which the alimentary canal is merely lobed, and the ctenophoral ribs are very much reduced in length; and by the fact that a Rhabdocoele is known, *Monotus*, which still, in the adult condition, carries an otocyst above the ganglion, as do Ctenophores.

Of course there are differences. Thus there are eight, not four, macromeres, in Ctenophora, and according to the best account we have of their development the ectoderm is separated into two sets of micromeres, not three sets.

But Surface has brought out clearly a hitherto unsuspected agreement between the two groups, viz. the origin of most of the so-called mesoderm. This material is really similar in both groups, consisting of stellate cells with processes, some of which are contractile. In both groups it results principally from cells budded from the macromeres, after the ectodermal material has been separated off; and the small cells budded from the lower poles of the macromeres of Ctenophora, after they have been almost covered by ectoderm, may well be compared to the fourth quartette of the Polyclade.

If we then accept provisionally this theory of Lang's, we are led to an interesting conclusion. We saw (p. 51) reason to assume the existence of a free-swimming, blastula-like, ancestral form, of which the Porifera were concluded to be degenerate sessile descendants. In the same way, the development of a gastrula from the blastula was shown to be evidence that the whole group of the Coelenterata are descendants of this pelagic group. Of this pelagic group, when it had attained the Coelenterate stage of development, the greater portion, as we saw in the last chapter, took to a sessile life, but one portion, the ancestors of modern Ctenophora, retained their free-swimming life. We now conclude that from this group of what we may term primitive Ctenophora, a set of degenerate descendants arose which gave rise to the Platyhelminthes.

The history of the Metazoa, so far as we have yet traced it, is that of a main pelagic group increasing in complexity of structure as time goes on, and at each level throwing off creeping and sessile stocks which are more or less degenerate in structure. We have indicated that the Polyclada are the only group of Platyhelminthes from the study of whose development much light can be expected on the origin of the phylum; but our knowledge is extremely defective even of the development of the Polyclada.

The student here meets with a phenomenon which will recur throughout the course of his study of Invertebrate Embryology, and it is this, that while the early development of the egg up to the period when the embryo becomes free, is known with some accuracy, next to nothing is known of the larval and post-larval stages. The reason for this ignorance lies chiefly in the difficulty found in obtaining appropriate food for the larva when the embryonic stores of nourishment are exhausted. This difficulty has been overcome in the case of Echinodermata, and further study will, no doubt, enable us to overcome it for the other groups also.

As a consequence of this state of affairs, however, there are vast gaps in our knowledge of the development of every group of Invertebrates; these gaps can and should be filled up. They offer a most promising field for further work. Thus, for example, in the development of *Polyclada*, we desire to know the origin of the extraordinary excretory system which is so characteristic of Platyhelminthes and, so far as we know, entirely unrepresented in any Ctenophore; also a knowledge of the development of the genital cells and of the genital ducts would be of extreme interest.

On the subject of the development of the excretory system Lang has a few observations. He has observed two ectodermic ingrowths at the sides of the embryo anteriorly, and these he looks upon as the rudiments of the main excretory canals; but we ought to have a detailed knowledge of their development.

When the development of *Polyclada* is thoroughly known the embryonic development of the other groups of Platyhelminthes will become of greatly increased interest, for it will show what alterations in a known type are produced by access to stores of food, and in this way throw light on the laws of variation, the ascertaining of which constitutes one of the root problems of the Science of Biology.

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CHAPTER VI

NEMERTINEA

Classification adopted

Protonemertini
Mesonemertini
Metanemertini
Heteronemertini

THE group of worms known as the Nemertinea constitute a very interesting division of the animal kingdom. They used to be regarded as a subdivision of Platyhelminthes, with the more primitive members of which they agree in possessing a soft ciliated skin. They also agree with Platyhelminthes in the character of their excretory system and in the general structure of their nervous system, but they differ profoundly from all Platyhelminthes in possessing an **anus.** The acquisition of a second opening to the alimentary canal and the consequent separation of the functions of ingestion and egestion, or defaecation, is a great structural advance, and is a reason for regarding Nemertinea as standing on a much higher level than Platyhelminthes.

The Nemertinea are divided by Bürger (1895) into four groups, Protonemertini, Mesonemertini, Metanemertini, and Heteronemertini. The development of the members of the first two divisions is quite unknown. Metanemertini pass through a shortened development within the egg-shell, the details of which have not been worked out owing to the extremely minute size of the eggs. The majority, however, of Heteronemertini develop into a free-swimming larva which, after leading a free life in the sea for some time, undergoes a remarkable metamorphosis into the adult worm. There are two types of this larva known, one termed the **Pilidium** and the other the **larva of Desor.** But the larva of Desor is quite evidently a simplified form of Pilidium, modified for a creeping rather than for a swimming life, and the Pilidium must be looked upon as the typical larva of the group.

CEREBRATULUS LACTEUS

We select therefore for special description the American species *Cerebratulus lacteus,* the eggs of which develop into a typical Pilidium.

The development has been worked out by C. B. Wilson (1900), and later by E. B. Wilson (1903), but similar species exist in the Mediterranean with very similar development, and have been worked at by E. B. Wilson's pupils, Yatsu (1904) and Zeleny (1904).

The egg of *Cerebratulus lacteus* whilst still in the ovary has a loose glassy membrane which at one end is drawn out into a point and thus offers a landmark in the egg; after the egg has been laid the spindle for the formation of the first polar body is formed, and this causes a little protuberance at the opposite pole of the egg. Unless fertilization supervenes the spindle is never completed, but when fertilization takes place the first and then the second polar bodies are nipped off and the loose glassy membrane is dissolved.

The egg divides into four macromeres so exactly similar to one another that it is quite impossible to distinguish an A, a B, a C, and a D segment, consequently the naming of the quadrants in the segmenting egg is an arbitrary matter. From these four macromeres quartettes of micromeres are budded off—the first quartette dexiotropically, the second laeotropically, until no less than six quartettes have been formed (Fig. 93).

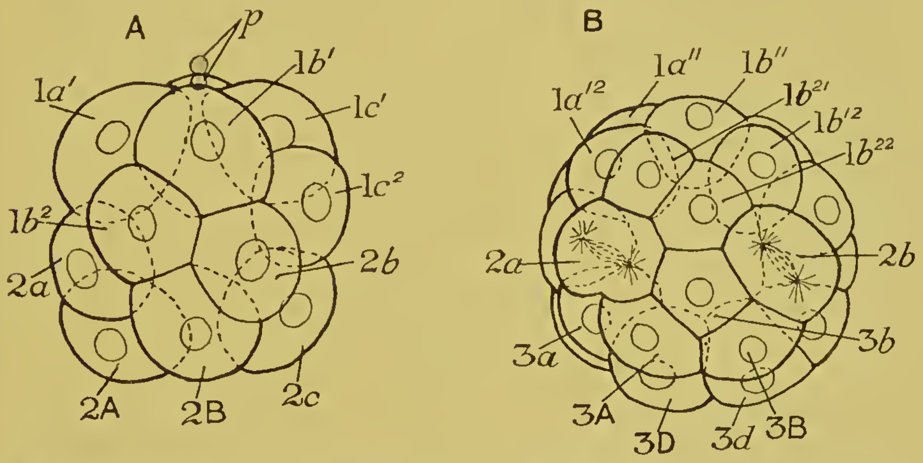

Fig. 93.—Two stages in the segmentation of the egg of *Cerebratulus lacteus* viewed from the side. (After E. Wilson.)

p, polar bodies. A, 16-cell stage. B, 28-cell stage passing into 32-cell stage.

The exact fate of these quartettes is not described in detail by Wilson. He remarks, however, that the endoderm extends to the equator of the spherical embryo. From this we infer that, as usual, the upper or ectodermic half of the segmented egg is constituted by the three first quartettes of micromeres; and that the fourth, fifth, and sixth quartettes, together with the residual macromeres, constitute the endoderm. The residual macromeres are similar in size and appearance to the last quartette of micromeres.

In this way a hollow sphere of cells, the **blastula**, is formed which is uniformly ciliated. Then one side of it becomes flattened and the cells here are somewhat larger than elsewhere. The whole shape of the blastula now resembled an obtuse cone.

Two large cells are budded into the cavity of the blastula, or blastocoele, one at each side of the flattened surface. These attach themselves by pseudopodia to various portions of the blastula wall. Then a patch of cells lying in the centre of the flattened surface is invaginated and forms a sac-like **gut**, whilst at the apex of the cone a thickening is formed which becomes slightly invaginated and forms a saucer-shaped depression. The cells forming this depression develop long stiff cilia, and in this way a characteristic **apical sense-organ** is formed. The blastula has thus become a **gastrula** (Fig. 94).

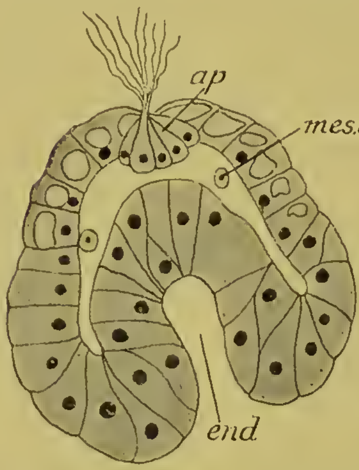

FIG. 94.—The young gastrula of *Cerebratulus lacteus.* (After C. Wilson.)

ap, apical plate; *end*, gut; *mes.ect*, mother cell of mesenchyme.

Each of the two large cells which passed into the blastocoele divides so as to give rise to a mass of branched cells, and these cells put out long pseudopodia which attach themselves to gut, to apical organ, and to skin, and some of these pseudopodia become converted into muscle fibres. Such cells are termed **mesenchyme.** The gut becomes differentiated into a sac-like globular stomach and a funnel-like oesophagus.

Round the edge of the flattened surface there is differentiated a thickened band of cells carrying specially long cilia. This band is called the "**prototroch,**" and it becomes the sole locomotor organ of the embryo, which now becomes a larva, escaping from the egg-shell and swimming about. The shell is broken by a spiral boring movement executed by the embryo. The prototroch is at first a simple circle, but it grows out into two lateral, downwardly-directed processes, like the ear-lappets of a policeman's helmet, and so the characteristic form of the Pilidium larva is attained (Fig. 95).

The principal muscles which have now been formed by the mesenchyme are as follows:—The retractor of the apical plate is a band of fibres which is attached above to the apical plate, and passes downwards splitting into right and left portions. These latter fibres are at first attached to the gut, but later, when the lappets are formed, they extend down into them, and their muscle fibrils extend from the oesophagus to the stomach, and from the post-oral ectoderm to the stomach; there is a strong sphincter muscle round the mouth. These muscles are all formed by processes of the larger mesenchyme cells. The smaller mesenchyme cells apply themselves to the inner surface of the ectoderm and to the outer surface of the gut. They give rise to a series of so-called "peritoneal" muscles which take the form of a fenestrated sheet. In the lappets the peritoneal muscles form radial muscles which serve to contract and elevate the lappets (Fig. 96).

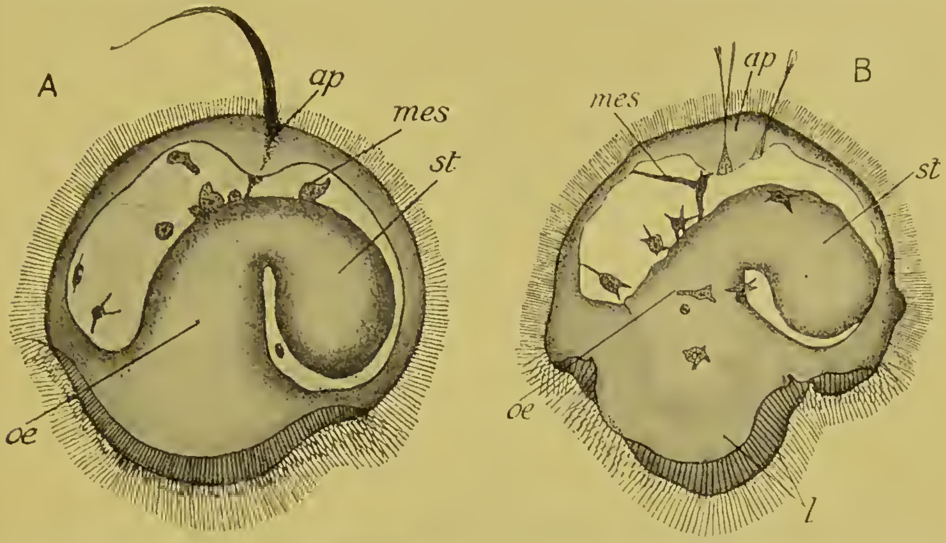

FIG. 95.—Two stages in the development of the Pilidium larva of *Cerebratulus lacteus*. Showing the development of mesenchyme into muscles. A, Earlier stage. B, Later stage. (After C. Wilson.)

l, lappets of prototroch; *oe*, oesophagus; *st*, stomach. Other letters as before.

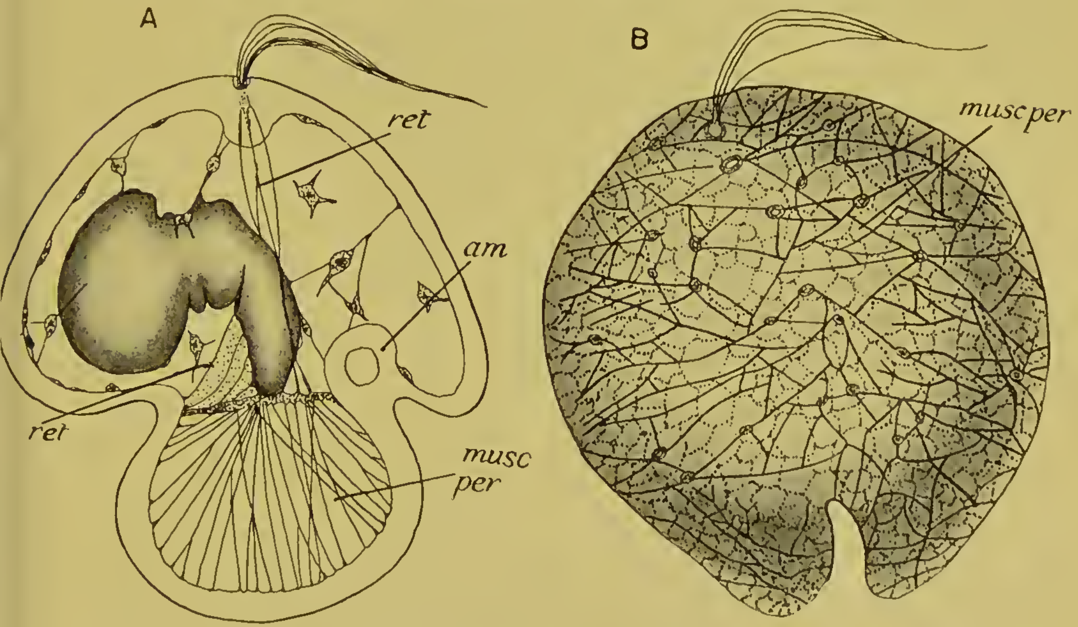

FIG. 96.—Two views of advanced Pilidium larva of *Cerebratulus lacteus* to show the development of the muscles. (After Wilson.)

A, viewed as a transparent object. B, surface view. *am*, anterior amniotic invagination; *musc.per*, so-called peritoneal muscles; *ret*, retractor of the apical plate.

On the posterior wall of the oesophagus a groove for conducting food appears, and numerous gland cells appear all over its wall.

Running round the edge of the larva under the prototroch a nerve-ring has been detected.

The development of the Pilidium is now complete, and it swims about at the surface of the sea feeding on microscopic organisms which are whisked into its mouth by the action of its cilia. After about two weeks it begins its metamorphosis. This has been described by Metschnikoff (1869) and Salensky (1886).

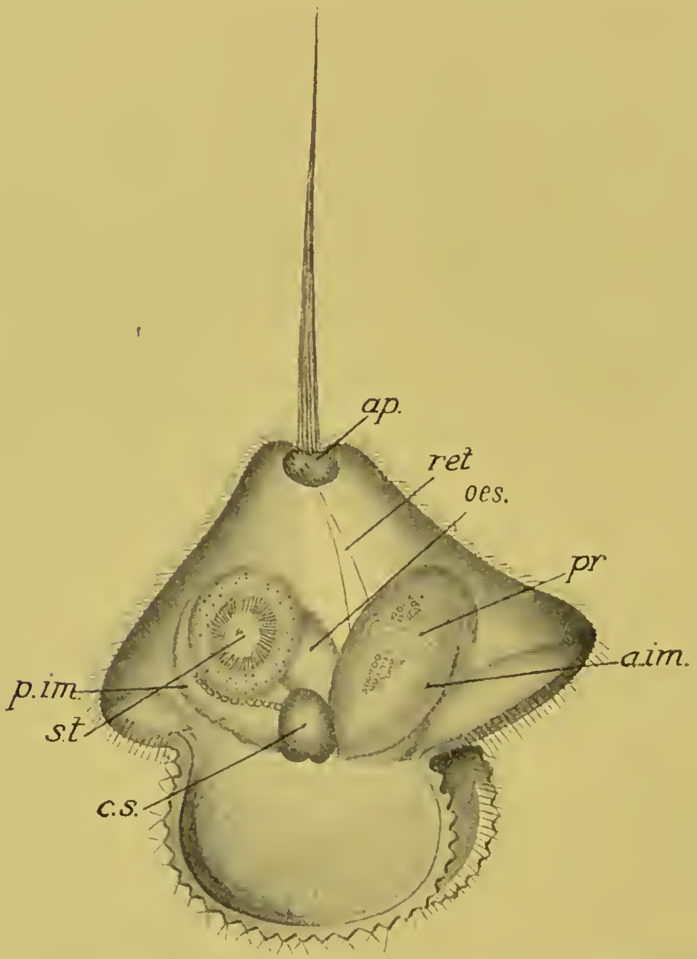

FIG. 97.—A Pilidium larva shortly before its metamorphosis. (After Metschnikoff.)

Letters as before. In addition, *a.im*, anterior imaginal disc; *c.s*, rudiment of cephalic slit; *pr*, rudiment of proboscis; *p.im*, posterior imaginal disc; *oes*, oesophagus.

On the flattened under side four ciliated invaginations of the ectoderm are formed. These are termed the **amniotic invaginations**, and their deeper portions are the **imaginal discs**. Two of these invaginations are situated opposite one another on the right and left sides of the animal respectively, in front of the mouth, and two others are similarly situated behind the mouth. Each of them grows and deepens, extending upwards over the surface of the globular stomach. Finally they meet one another, fuse and coalesce, the anterior and posterior on each side and the right and left on each side. The imaginal discs form the skin of the future worm whilst the outer walls of the coalesced invaginations form a temporary envelope known as the **amnion.**

Before coalescence is quite complete the organs of the future worm are constructed, and as to the manner in which this is accomplished we have tantalizingly little information. It appears from Salensky's account (1886) that the skin of the anterior part of the animal, as far back as the cephalic slits, originates from the anterior imaginal discs. The posterior imaginal discs form the skin of the hinder part of the body of the worm. The characteristic **proboscis** is formed as an ectodermal invagination. The **proboscis sheath** originates as a solid mass of mesoderm into which the proboscis invagination projects (Fig. 99). This mesoderm appears to be in close proximity to the ectodermal wall of the posterior imaginal disc on each side and possibly arises from it. Later the rudiment of the sheath becomes hollowed out and forms a sac lined by flattened cells and filled with fluid. The adult **brain** (*br*, Fig. 99) arises as a thickening of the ectoderm of the anterior imaginal discs. The **cephalic slits** likewise arise as ectodermal ingrowths, not from the imaginal discs but from the larval ectoderm between anterior and posterior discs, and pouches grow out from the oesophagus to meet them (*oe.p*, Fig. 99 A). An anus must be formed, but as to how or when we have no information.

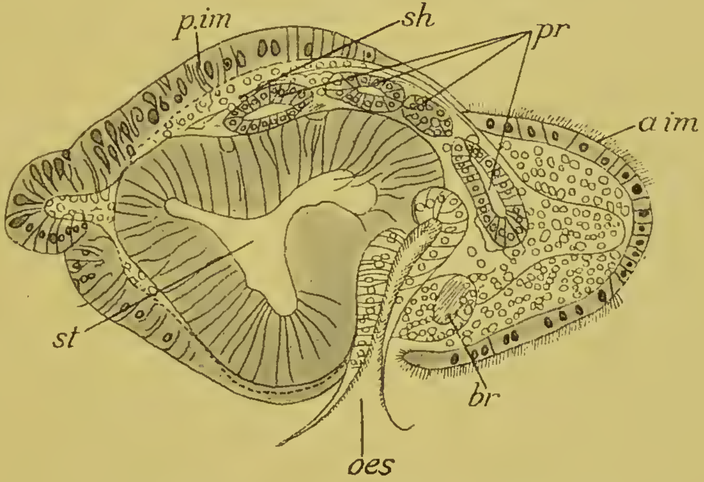

FIG. 98.—Longitudinal section through a Pilidium larva of about the age of that represented in Fig. 97. (After Salensky.)

br, rudiment of brain; *sh*, rudiment of sheath of proboscis.

In fact nearly all our information about this period of development is based on the examination, as whole objects, of larvae fished from the sea, although Salensky has to some extent applied the method of sections. If once an appropriate food for the Pilidium larvae could be discovered, so that these larvae could be reared in large numbers through their metamorphosis, under experimental conditions, and if each stage in this change were thoroughly examined by sections, then a flood of much-needed light would be thrown on this period of Nemertine development.

If the reader has followed the description so far given it will be evident that when all four amniotic invaginations completely coalesce they must cut the larva into an upper and a lower half. This is just what happens; and the lower and inner half, invested by the coalesced floors of the amniotic invaginations, and containing the alimentary canal, drops to the bottom of the sea and commences life as a young Nemertine worm. The upper half consisting of the larval ectoderm, including the prototroch, lappets, and apical sense organ, and bounded inside by the coalesced roofs of the invaginations or amniotic investment, continues to swim about for a little time before its energies are exhausted, and then it dies.

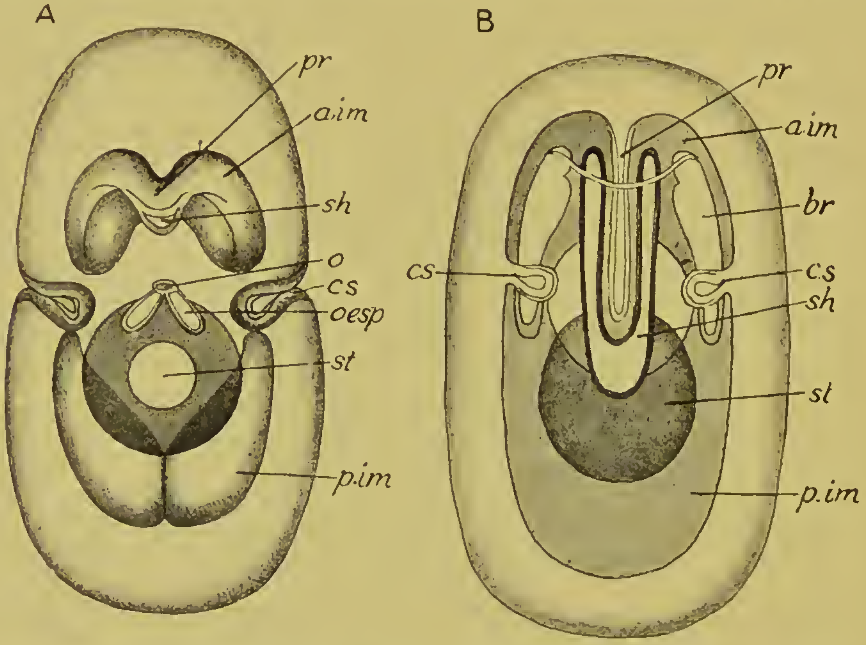

Fig. 99.—Two stages in the development of the Nemertine rudiment within the Pilidium, viewed from above. (After Salensky.)

c.s, cephalic slits; o, mouth; oes.p, oesophageal pockets.

EXPERIMENTAL WORK.

E. B. Wilson and his pupils Yatsu and Zeleny have performed a most interesting series of experiments on the eggs and embryos of *Cerebratulus*, the general results of which may be shortly recounted here. The unfertilized egg was cut or shaken into fragments. If this be done before the membrane of the nucleus has disappeared, and if sperm be added to the fragments, only the fragment in which the nucleus is situated develops into a larva. But if the same experiment be performed after the nuclear membrane has faded, all the fragments will develop into larvae. It is, therefore, obvious, that when the nuclear membrane fades, some substance must pass into the cytoplasm which confers on any fragment of it the power to develop into a larva if a spermatozoon be added to it.

If the same experiment be performed after normal fertilization has occurred, only the fragment containing the first invading spermatozoon will develop. All attempts to fertilize the other fragments by adding fresh spermatozoa failed.

In the majority of cases the developing fragment is the one containing the zygote nucleus; but in some cases, when the fragmentation of the egg had occurred before the spermatozoon had reached the nucleus, it is the fragment containing the spermatozoon and not that containing the nucleus which develops, while the fragment containing the latter can be seen to form the polar bodies, but it goes no farther in development.

Therefore, just as some substance must exude from the egg nucleus which confers on all the cytoplasm the power to form a larva, so we are bound to conclude that some material is given off from the sperm head which inhibits development in the cytoplasm, except when under the influence of the first nucleus.

When the egg was cut into fragments, however, and a piece was induced to develop, it gave rise to a perfect Pilidium larva of correspondingly reduced size. The segmentation occurred as in the normal larva, though the blastomeres were correspondingly smaller. But when the first two blastomeres of a normal egg were separated from one another, each divided as if it still formed part of the whole egg,—it formed two macromeres and two micromeres. The separation was effected by exposing the developing eggs to the influence of artificial sea-water, made up so as to entirely exclude lime; such water causes the blastomeres to lose their adhesion to one another and to fall apart, owing apparently to an alteration in the physical characteristics of the outermost layer of the cytoplasm. The separated blastomeres are then restored to normal sea-water and allowed to continue their development.

When one of the first four blastomeres is separated it forms one macromere and one micromere by the first division, and continues to segment as if it formed one-fourth of the egg. Nevertheless in both these cases the half or quarter blastula closes its wound by narrowing and contraction of the edges, and develops into a Pilidium which is perfectly normal but of reduced size. The Pilidium which develops from one of the first four blastomeres, however, has its apical plate displaced forwards, a change which is probably due to the size of the cells, derived from the segmentation of the blastomeres, remaining the same as if they still formed part of a whole egg. Each cell has therefore to form a part of the larva proportionally four times as great as it would normally have done, and so it must be subjected to much more severe curvature than usual, and these curvatures produce a series of strains which distort the resulting larva.

A cell of the 8-cell stage is incapable of developing into a Pilidium. When the 8-cell stage is broken in two, its two constituent portions, viz. the macromeres and the first quartette of micromeres, each group of four cells can develop into a larva. But the micromeric group form a larva with a very large apical sense-organ and no gut, whilst the macromeric group develops into a larva with an enormous gut and no apical organ. The same result is obtained by cutting the blastula along the equator, in this case the upper half forms a larva with enormous apical organ and vestigial gut, whilst the lower half forms a larva with large gut and no apical organ. Hence we conclude that whereas every one of the first four blastomeres contains all the substances necessary to form a perfect larva, after the occurrence of the third cleavage the substance necessary for the formation of the gut is restricted to the lower cells, whilst that destined to form the apical organ is confined to the upper four cells.

Yatsu found that when the fertilized egg is cut into fragments abnormal Pilidia are produced, except where only a small fragment from the animal pole has been removed, and hence he concludes that the material destined to form the apical plate is situated not at the animal pole but in a ring a short distance beneath it.

When we review the results of these experiments we are struck with the demonstration which they afford of the influence of the materials given off from the nuclei on the cytoplasm, and also with the proof that at the moment when sperm and egg nuclei approach one another a definite structure or arrangement of organogenetic materials is impressed on the cytoplasm. The outward and visible sign of this inward process may be the radiations which extend from the sperm nucleus outwards. This conclusion will be supported by evidence to which we shall call attention during our study of various other invertebrate groups. The structure impressed on the cytoplasm reminds us of what was found to be the case with the Ctenophore egg, but it is not so definitely specialized as in the Ctenophore egg. In this respect the egg of the Nemertine occupies an intermediate position between the egg of the Hydromedusan and the egg of the Ctenophore.

AFFINITIES OF NEMERTINEA.

We now approach the final question as to what light the development of *Cerebratulus* throws on the ancestry of the Nemertinea as a whole. This question resolves itself into the problem: What is the ancestral significance of the Pilidium larva? We have to interpret a larva with a simple sac-like gut, opening by a mouth at its lower pole, whilst its upper pole is occupied by a cup-like sense-organ carrying long stiff cilia, and its locomotion is effected by a lobed band of cilia. Just as in the case of Müller's larva we are again reminded of a primitive Ctenophore. Müller's larva does not carry the apical tuft of hairs, and in this respect is less like a Ctenophore than the Pilidium larva; but in having its ciliated band produced into eight processes instead of two, it is more like a Ctenophore than the Pilidium. We probably shall not go far astray in concluding that the Pilidium represents a free-swimming ancestor of the Nemertinea, belonging to the same great group as that containing the ancestor of the Ctenophores, but differing from the latter as a shark differs from a salmon, whilst both are fish.

The metamorphosis into the Nemertine worm must be regarded as the immensely shortened recapitulation of the long development which occurred before this ancestor developed into a Nemertine; a development which must have been much longer than that which was necessary to convert the ancestor denoted by Müller's larva into the Polyclade worm. Proof will be given as our studies proceed, that such a **cataclysmic metamorphosis** as that of the Nemertine has been secondarily derived from a type of development that was originally slow and gradual. Nevertheless, as we have indicated above, if this metamorphosis were thoroughly studied in detail we should know a great deal more about the steps by which that change was accomplished than we do at present.

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CHAPTER VII

ANNELIDA

Classification adopted

- **Archiannelida**
- **Chaetopoda**
  - **Polychaeta**
    - **Nereidiformia**
    - **Spioniformia**
    - **Terebelliformia**
    - **Capitelliformia**
    - **Scoleciformia**
    - **Scabelliformia**
    - **Hermelliformia**
  - **Oligochaeta**
- **Hirudinea**
  - **Acanthobdellidae**
  - **Rhyncobdellidae**
  - **Gnathobdellidae**

The group of segmented worms known as Annelida has furnished subjects for an immense amount of embryological study, but there are a great many points in their development still unsettled which offer a wide field for future research. Although widely diverse from each other in their adult structure the members of the group show a remarkable uniformity in their early development, so that the complete description of a single type will serve as a guide to what is known about the development of all.

Annelida are divided into **Archiannelida**, including *Polygordius* and a few allied forms which never develop chaetae and are devoid of external circular muscles; **Polychaeta**, the central group, including worms with numerous chaetae, well-developed parapodia, and external circular muscles; **Oligochaeta**, freshwater and terrestrial worms, with few chaetae, complicated genital organs, no parapodia, but provided with external circular muscles; and **Hirudinea**, extremely modified forms with obscure segmentation, no chaetae or parapodia, but with external circular muscles, extremely complicated genital organs, and suckers used for progression.

Of these forms the most primitive, and the one which shows the longest larval development, is the Archiannelidan *Polygordius*. The embryology of this form has been worked out in great detail recently by Woltereck (1902, 1903, 1905), and we select it as type for special description. As, however, although *Polygordius* occurs on both sides of the Atlantic and in both North Sea and Mediterranean, it is not very abundant or easy to obtain, some practical directions will be given as to the means of dealing with the eggs of *Pomatoceros*, a very common Polychaete belonging to the family Serpulidae. The development of *Pomatoceros*, in the early stages at least, is almost identical with that of *Polygordius*, and in one or two points even more primitive.

The eggs of all Annelida undergo cleavage of the spiral type, which we have already studied in the case of the Platyhelminth *Planocera*. In Annelida, as in *Planocera*, the ectoderm is separated as three successive quartettes of micromeres. As in *Planocera* also, a blastula consisting of relatively few cells is formed, which, by invagination or **epibole** (see p. 92) is converted into a gastrula.

METHODS

Now for the study of such eggs the method of sections is of very little use. This method requires that the egg to be studied should consist of a large number of similar cells, so that a sample such as a section presents would give a good idea of the whole; but where the egg consists of relatively few cells and these are individualized at an early stage of development the method obviously fails. So there is nothing left but to make whole mounts and endeavour (as Surface did in the case of *Planocera*) to identify and trace the history of each individual blastomere.

This procedure, as already mentioned (Chap. V. p. 104), is termed the study of **Cell-lineage**; it was introduced by the American zoologist Whitman (1878), who first employed it in the study of the eggs of Hirudinea, and it was taken up by a brilliant school, which Whitman founded, one of the most prominent of which was Prof. E. B. Wilson (1892).

Prof. Wilson applied the method to the study of the development of the Polychaete *Nereis*, a work which threw much light on Annelidan embryology. Other pieces of work of equal merit were those of Treadwell on *Podarke* (1901) and Child on *Arenicola* (1910).

If, nevertheless, we select the work of a German for special description, when the credit of most of the investigations belongs to Americans, it is solely because the development of the type on which he worked is so primitive and simple that, once it is known, all the others can easily be described in terms of it.

In order that the cells may be identified in whole mounts of eggs, it is necessary that these should be rendered transparent, and that they should be examined from all sides. As the eggs of many species are opaque owing to the fact that they contain numerous yolk grains, this is not easy to do. Prof. E. B. Wilson employs a mixture of 3 parts of glacial acetic acid and 1 part glycerine. This mixture in many eggs dissolves the yolk granules and makes the whole of a glassy transparency. The preparations made in this way, however, are not permanent, but they last long enough to enable good drawings to be made. Other authors make permanent preparations by preserving the eggs in "Eisig's mixture," *i.e.* 3 parts of saturated aqueous solution of corrosive sublimate and 1 part of glacial acetic acid, staining with haematoxylin and trusting, after dehydration by alcohol, to oil of cloves to clear them sufficiently to allow of complete examination.

In order to examine the eggs from all sides Wilson rolls them about on the slide by moving the coverslip, which he supports on feet made of a mixture of beeswax and vaseline, the proper height of which can be ascertained by trial. Other workers attain the same end by introducing between slide and coverslip a piece of thin capillary glass rod or tube, drawn out to the requisite degree of tenuity.

When the segmentation is completed the embryo issues from the egg membrane and commences to lead a free life as a larva. The form of this larva resembles in broad outline the form of the Pilidium. Like it, it possesses an apical plate with a tuft of long cilia and a prototrochal girdle, and it is called a **Trochophore.**

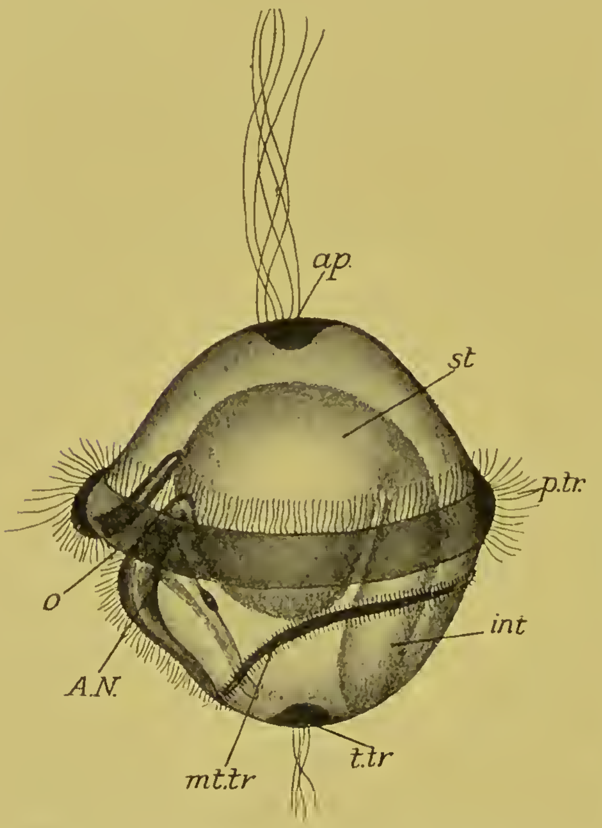


FIG. 100.—The Trochophore Larva of *Polygordius*, viewed from the side. (After Woltereck.)

A.N, archinephridium; *ap*, apical plate; *int*, intestine; *mt.tr*, metatroch; *o*, mouth; *p.tr*, prototroch; *st*, stomach; *t.tr*, tetotroch.

The Trochophore differs from the Pilidium in possessing an intestine terminating in an anus, and in having a post-trochal region of the body which projects behind the prototroch, instead of having only a concave surface in this position such as is found in the Pilidium. By the gradual growth and elongation of the post-trochal region, the body of the worm is formed.

In the case of the Serpulid *Pomatoceros* the eggs and sperm are easily obtained by simply extracting the animals from their tubes and placing them in clean sea-water. If the genital cells are ripe they will immediately be shed, and in this way a natural fertilization of the eggs is accomplished.

The Trochophore issues on the second day and rises to the top of the water. It can be reared through its entire development by supplying it with a pure culture of the diatom *Nitschia*. Such pure cultures can be obtained from Dr. Allen, Director of the Marine Biological Station at Plymouth, and they serve as pabulum for many different kinds of larvae.

Pure diatom cultures were obtained originally by isolating under the microscope a single individual of the species of diatom desired, and then transferring it to a flask of sterilized and filtered sea-water. The sea-water is first shaken up with animal charcoal and decanted in order to remove all soluble toxins, and then passed through a Berkfeldt stone filter, which removes all organisms, even bacteria. To the sea-water is now added a certain amount of Miguel's solution, about 2 drops per 100 c.c. of water, and the flask is stopped by a plug of sterilized cotton wool. In a month's time a copious growth of the desired diatom is obtained.

If a pipette-full of such a culture be added to an evaporating dish containing the larvae of *Pomatoceros*, these will develop normally and eventually metamorphose into the adult worms, which form tubes and attach themselves to the sides of the glass. In this way the whole life-cycle can be controlled, and such larvae can be examined living, or mounted whole, or examined by sections.

The fixative found best is Eisig's mixture (see *ante*). The methods of orientating, embedding, and cutting have been fully described in Chapter II.

POLYGORDIUS. CELL-LINEAGE

Returning now to *Polygordius* we should remind the student that this is a minute worm which burrows in mud and sand. The eggs are excessively minute and very transparent, and the segmentation is remarkable for its extreme regularity. The eggs are dehisced into the sea by the breaking up of the parent's body and are fertilized there.

Up to the 64-cell stage all the cells divide at the same time, so that we have successive "**cleavages**" which successively divide the egg into 2, 4, 8, 16, 32, and 64 cells, that, is six cleavages in all. Moreover a 128-cell stage is very nearly realized, for all the cells of the 64-cell stage divide nearly synchronously, except those forming the prototroch and a few others of the upper hemisphere, which, having reached the summit of their development, divide no more. The macromeres are all precisely equal in size; it is therefore at first impossible to discriminate an A from a B, a C, or a D (see Chapter V.), but in the later cleavage stages this can be done, owing to the different way in which members of the second and third quartettes of micromeres, given off from the different macromeres, behave.

Shortly after the 64-cell stage has been reached cilia appear on the cells destined to form the prototroch (Fig. 101, B). The embryo then begins to rotate within the vitelline membrane, which it soon ruptures, and it then begins its free-swimming existence.

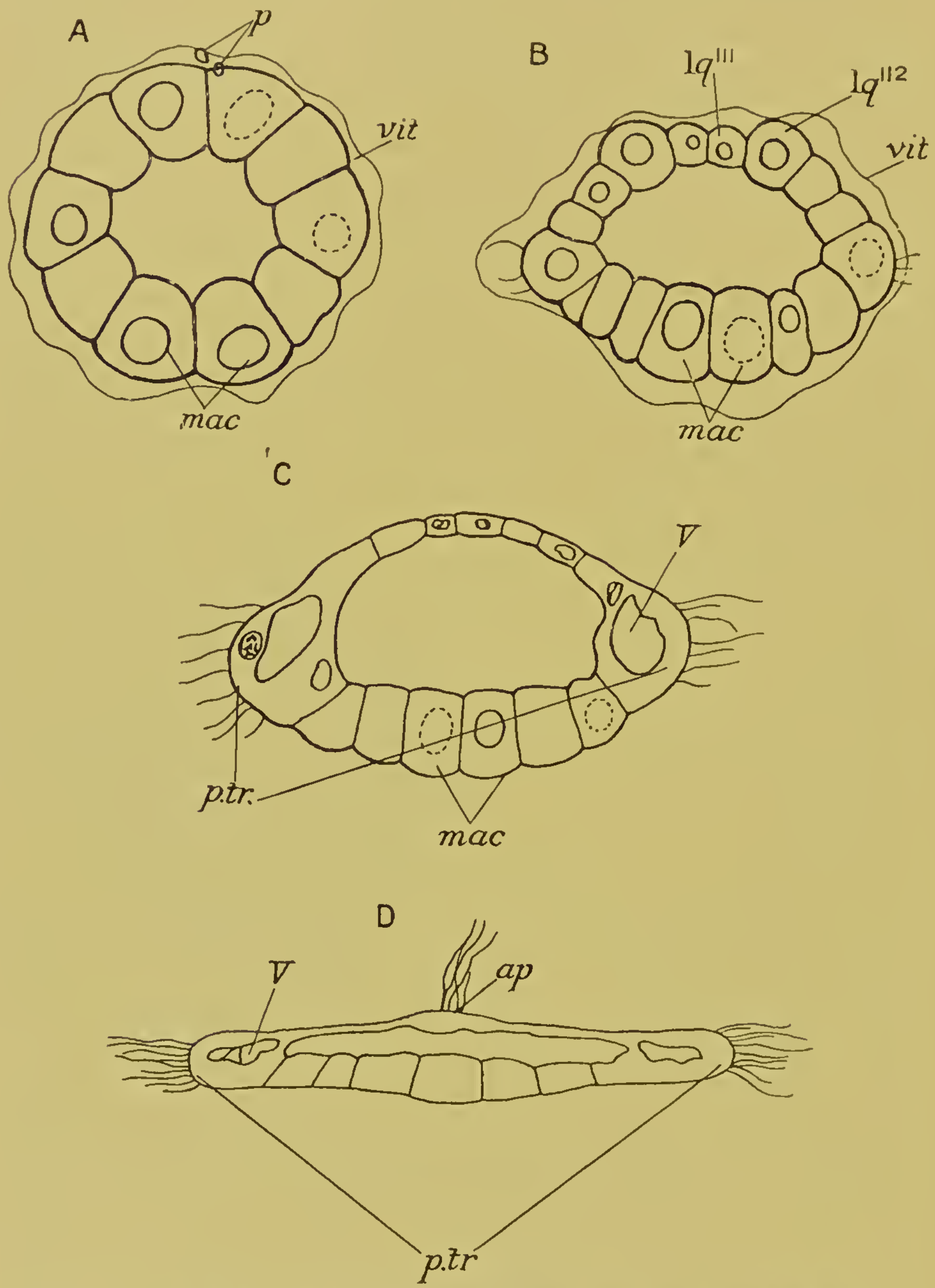


FIG. 101.—Stages in development of the blastula of *Polygordius* seen in optical longitudinal section.

A, 32-cell stage; B, 64-cell stage; C, 76-cell stage; D, 116-cell stage. Letters as before. In addition, *mac*, residual macromeres; *p*, polar bodies; *v*, vacuoles in cells forming the prototroch; *vit*, vitelline membrane; $1q^{111}$, the apical cells; $1q^{112}$, the mother cells of the Annelidan cross.

When about 140 cells have been formed the fully-segmented egg constitutes a thick-walled, extremely flattened **blastula** which is converted into a **gastrula**, and then into a Trochophore, by rearrangements of cells, without further cell-division. Further divisions of cells only occur after the Trochophore has been feeding for some time.

Up to the 64-cell stage then, all the cells of the egg divide simultaneously, so that the first micromeres divide once as the second quartette of micromeres is given off. When the division to form the third quartette of micromeres is complete, the first quartette have divided twice; and when the fourth quartette, which gives rise to mesoderm and endoderm, is formed, they have divided thrice, and with this division the 64-cell stage is attained.

The egg, which from the 16-cell stage had taken on the form of a hollow spherical blastula, now begins to flatten out. In the same period the second quartette of micromeres has divided twice and the third quartette once.

At the next cleavage the cells of the prototroch fail to divide and so do certain other cells, descendants of the first group of micromeres, but all the other cells of the egg divide. A fifth quartette is given off, which is destined to form endoderm only, and the residual macromeres are now barely if at all larger than the micromeres to which they gave rise. The flattening of the embryo continues till it assumes the form of a flattened plate (Fig. 101, D).

The next cleavage is participated in only by the macromeres, which divide not spirally but symmetrically with regard to the future median plane of the embryo. The fifth quartette of micromeres also divide, so do the fourth, and some cells of the second and third; and then invagination commences.

In describing in detail the divisions of the cells it is most convenient to deal with the different quartettes of micromeres separately. Since, as has been already stated, it is impossible in the earlier divisions to distinguish one macromere from another, it is convenient to be able to refer to them collectively, and the letter q is used to denote a, b, c, and d.

In all four quadrants of the egg the divisions of the first quartette of micromeres are exactly alike. We say that 1q divides into upper cells $1q^1$, mothers of the whole upper hemisphere of the Trochophore above the prototroch, and into lower cells $1q^2$, the mothers of the prototroch; $1q^1$ divides again into upper cells $1q^{11}$, mothers of the apical plate, or as it is sometimes termed the **rosette**, and of a St. Andrew's cross of cells radiating from it, called the "**Annelidan cross**" because it is conspicuous in the eggs of all Annelids; and into lower cells $1q^{12}$, mothers of a group of cells called by Wilson the **intermediate girdle cells** but termed by other authors the **Molluscan cross**, because in Molluscan eggs these cells take on the form of a conspicuous upright cross. $1q^2$ divides into $1q^{21}$, and $1q^{22}$, one set of cells lying obliquely above the other.

At the next cleavage $1q^{11}$ divides into $1q^{111}$, forming the **apical cells** or **rosette** which carry the tuft of long stiff cilia which constitutes the apical sense-organ, and into 1q^{112}, which form the rudiment of the **Annelidan cross.** 1q^{112} bud off cells between themselves and the apical cells, and in this way the arms of the cross are formed, that is to say, 1q^{112} divide into 1q^{1121} and 1q^{1122}.

Turning now to the other cells of the upper hemisphere we find that 1q^{12} behaves similarly. It divides into 1q^{121} and 1q^{122}, and 1q^{121} further divides into 1q^{1211} and 1q^{1212}. These three cells in each quadrant, 1q^{122}, 1q^{1212}, and 1q^{1211}, are in four curved series, and this is also true of primitive Mollusca, but in higher Mollusca they are arranged in four straight lines and form the upright cross mentioned above.

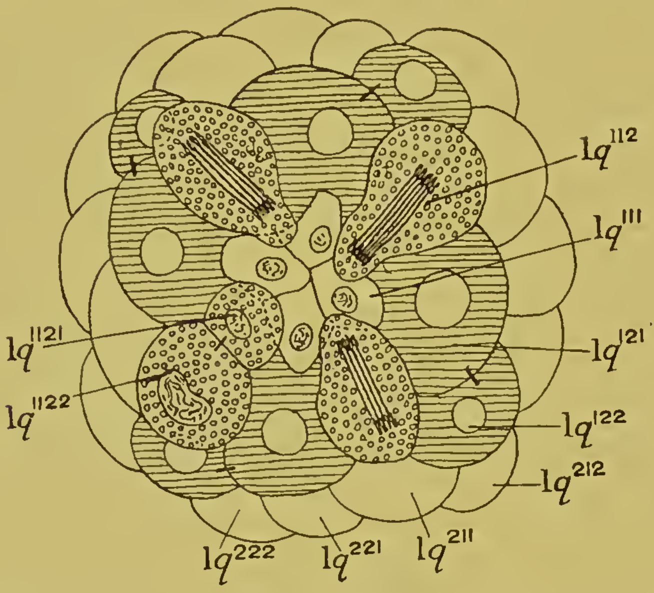


FIG. 102.—Dorsal view of upper hemisphere of egg of *Polygordius*, in which seventy-six cells have been formed.

The rosette cells and the cells of the prototroch are left clear. The cells of the "Molluscan" cross are cross-hatched. Those of the "Annelidan" cross are marked with circles.

The group of cells 1q^{21} and 1q^{22} each divide into two sets of cells, so that we have four daughters of 1q^{2} in each quadrant; and these sixteen cells acquire long powerful cilia and constitute the **prototroch.** They become large, clear, and vacuolated, and at first—and this is most interesting and important—*they form four discrete groups*; it is only later that these groups coalesce to form a complete ring.

If this description has been followed it will be seen that the upper hemisphere of the egg, including the prototroch, when divisions temporarily cease, consists of forty cells. In the 64-cell stage it consists of course of thirty-two cells, since it forms exactly half the egg, but the cells constituting the "Annelidan" and "Molluscan" crosses divide once again, and this brings the total number of cells up to forty.

When we turn the flattened embryo over and view it from the vegetative pole, we are able, once the 64-cell stage has been passed, to distinguish the various quadrants of the egg from one another, and to tell which is A and which is B, which C and which D.

Now the embryo has a somewhat squarish outline and the rounded corners are formed by the groups of prototrochal cells belonging to the first quartette. These groups are directly opposite the respective macromeres from which they arose; for, if we take the group derived from A for example, all its members are daughters of $1a^2$ and ultimately of 1a. But 1a itself was given off dexiotropically from A; that is to say, lay above and to the right of it. The next division is a laeotropic one, that is to say, as the name implies, $1a^1$, the upper daughter, lies above and to the left of the lower daughter $1a^2$. Now this formation of the spindle with a left bend has the effect of causing $1a^2$ itself to pass somewhat to the left, and thus undo to a certain extent its original right-hand twist, so that it eventually comes almost exactly opposite A.

The line joining the prototrochal group of cells and the macromeres constitutes a **radius** of the figure, and cells or cell groups lying on this radius are said to be **radial**, and cells or cell groups alternating with them are said to be **inter-radial**. Now it is found that the third and fifth quartettes of micromeres are radial whereas the second and fourth are inter-radial. The following rule then is found to hold for the fate of cells forming these quartettes. In the radial quartettes the cells in quadrants A and B behave alike, but the cells in C and D while behaving like each other behave differently from those in A and B. In the inter-radial quartettes the cells in quadrants in A, B, and C, behave alike, but the cells in quadrant D behave differently from each of them.

Turning our attention now to the second quartette, and taking a as example for a, b, etc., we find that 2a divides into $2a^1$ and $2a^2$, one directly above the other. Each then divides laeotropically into $2a^{11}$ and $2a^{12}$, and $2a^{21}$ and $2a^{22}$, respectively, thus forming a lozenge-shaped group of four cells. Of these four the outermost divides radially into $2a^{111}$ and $2a^{112}$, lying almost side by side; whilst $2a^{12}$ and $2a^{21}$ divide obliquely into $2a^{121}$ and $2a^{122}$, $2a^{211}$ and $2a^{212}$ respectively. These cells help to form a belt of flat clear cells lying just beneath the prototroch, and they divide no more till the Trochophore stage is reached. The fate of the innermost cell $2a^{22}$ is different; it divides by a tangential cleavage into an outer cell $2a^{221}$ and an inner cell $2a^{222}$. Then each of these divides into a larger anterior cell $2a^{221a}$ and $2a^{222a}$ respectively and a small posterior cell $2a^{221p}$ and $2a^{222p}$ respectively.

Exactly the same division takes place in quadrant C, and then the small posterior cells sink into the blastocoele and form that part of the **mesectoderm** or **larval mesoderm**, which will eventually give rise to the circular and radiating muscles of the larval oesophagus.

In quadrant B, however, $2b^{221}$ divides into an outer and upper cell $2b^{2211}$, and an inner and lower cell $2b^{2212}$ whilst $2b^{222}$ divides into two sisters lying side by side, viz. $2b^{222r}$ and $2b^{222l}$. Now these four cells in quadrant B, and the two cells in quadrant A, viz. $2a^{221a}$ and

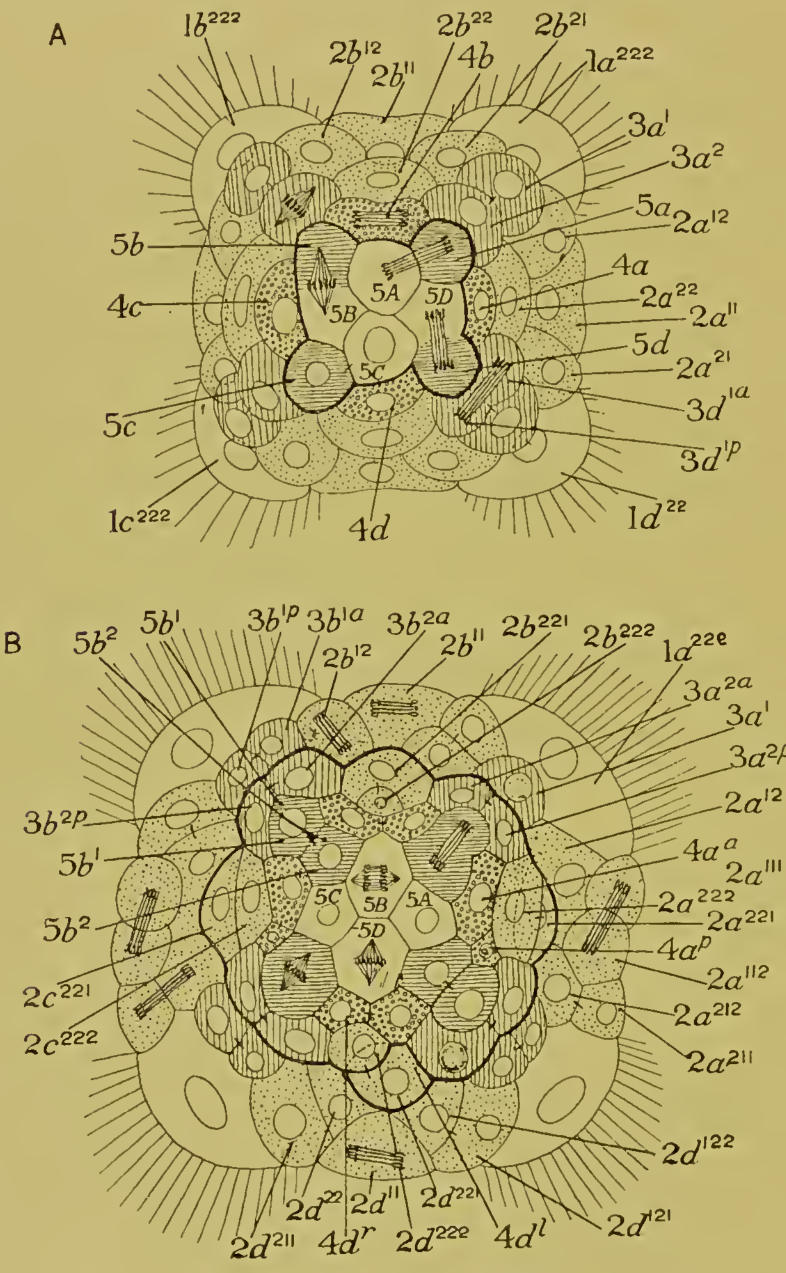


FIG. 103.—(*Continued on opposite page.*)

$2a^{222a}$, and in quadrant C, viz. $2c^{221a}$ and $2c^{222a}$ (Fig. 103, C), are destined to form the **stomodaeum**, but for the complete history of this structure we must wait until we have considered the history of the third quartette of micromeres.

In the quadrant D the divisions at this stage are similar, but $2d^{222}$ and $2d^{221}$ do not divide until the Trochophore stage is reached. It follows that the so-called larval mesoderm is formed from the second quartette in three of the four quadrants of the egg.

We now pass to the consideration of the third quartette, and we would remind our readers that this quartette is *radially* situated, whereas the one we have just been considering was inter-radial; and further, as in eggs with spiral cleavage in general, the second and third quartettes of micromeres come to lie about the same parallel of latitude, so to speak, on the globe represented by the whole egg, since the quadrants of one quartette occupy the gaps between the quadrants of the other.

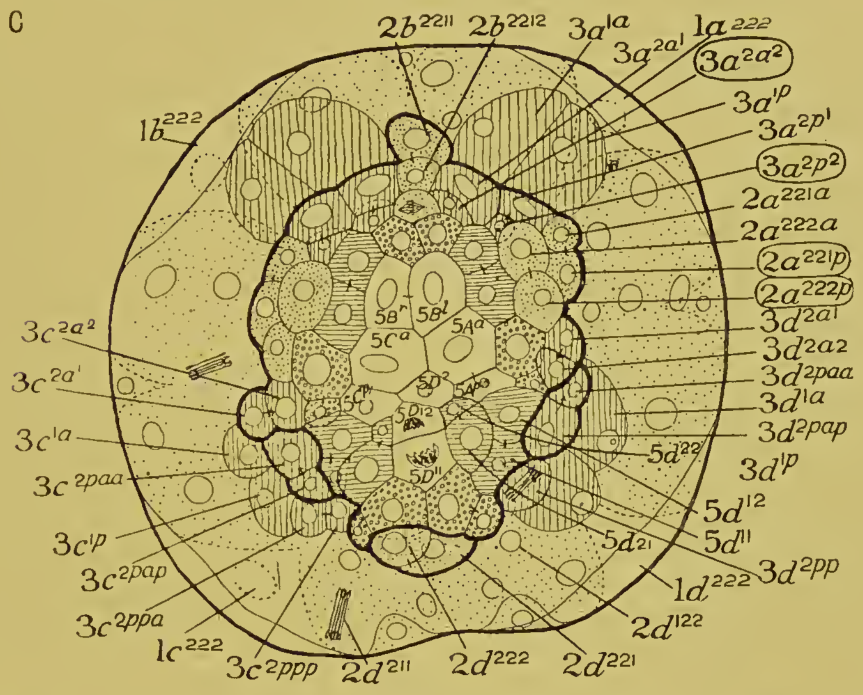


FIG. 103 (*continued*).—Three stages in the segmentation of the lower or vegetative surface of the egg of *Polygordius*.

A, stage of about 76 cells; B, stage of about 112 cells; C, later stage in which a mass of rapidly dividing cells at the lower pole is sharply distinguished from an outer zone of clear cells. The heavy black line surrounds the cells which later will take part in the process of invagination and the formation of the lips of the blastopore. The cells belonging to the second quartette are dotted, those belonging to the third quartette are marked by vertical lines. The cells belonging to the fourth quartette are marked by little circles, those belonging to the fifth quartette by horizontal lines. The residual macromeres, and those belonging to the first quartette, are left white. The names of the cells which form the larval mesoderm are surrounded by circles. Cf. ($2a^{221p}$).

Taking then the quadrant A first (and what applies to A is true also of B) we find that 3a divides into $3a^1$ and $3a^2$; and each of these now divides into an anterior and posterior cell, *i.e.* $3a^{1a}$, $3a^{1p}$, $3a^{2a}$ and $3a^{2p}$, respectively. Of these the first two $3a^{1a}$ and $3a^{1p}$, remain undivided and help to complete the belt of broad flat cells, the other parts of which are formed by the cells of the second quartette, to which allusion has already been made. The other two cells each divide into an anterior outer large cell, $3a^{2a1}$ and $3a^{2p1}$, respectively, and a posterior inner smaller cell, $3a^{2a2}$ and $3a^{2p2}$, respectively. The last two eventually sink into the blastocoele and help, like the similar cells of the second quartette, to form larval mesoderm, whilst their two larger sisters enter into the formation of the stomodaeum.

We are now able to take a more general survey of the cells which enter into the formation of this structure. The front wall of the stomodaeum is formed by the four cells $2b^{2211}$, $2b^{2212}$, $2b^{222r}$ and $2b^{222l}$. Its right side is constituted by the cells $2c^{221a}$ and $2c^{222a}$, and its left side by the corresponding cells $2a^{221a}$ and $2a^{222p}$. In its right anterior corner we find the cells $3b^{2a1}$ and $3b^{2p1}$, in its left anterior corner the corresponding cells $3a^{2a1}$ and $3a^{2p1}$.

In the quadrants C and D the micromeres of the third quartette divide, at first, similarly to those belonging to quadrant A, B. Thus, taking 3d for example (and remembering that all said about it is equally true of 3c), we find that it divides into $3d^1$ and $3d^2$, and each divides into anterior and posterior cells: of these $3d^{1a}$ and $3d^{1p}$ become broad and flat and remain undivided, and thus complete the band of this kind of cell right round the egg; though at a later period, as we shall see, they form part of the posterior lip of the mouth. $3d^{2a}$ divides into $3d^{2a1}$ and $3d^{2a2}$, and these cells will help to complete the hinder wall of the stomodaeum in a manner to be described later. $3d^{2p}$, however, divides into an anterior and a posterior cell, $3d^{2pa}$ and $3d^{2pp}$, and both these cells undergo another similar division, so that we get an antero-posterior directed line of four cells, $3d^{2paa}$, $3d^{2pap}$, $3d^{2ppa}$, and $3d^{2ppp}$ (Fig. 103, C).

The last two of these cells constitute the rudiment of one of the larval kidneys or **archinephridia**, the other being formed by the corresponding cells in quadrant C. The more anterior cell of each pair at a later stage sinks into the blastocoele and is transformed into a flame cell or **solenocyte**, with a cavity and a tuft of cilia waving within it; whereas $3d^{2ppp}$ forms the excretory tube and remains in connection with the ectoderm (Fig. 106).

Passing now to the fourth quartette we find that all four cells divide radially, each giving rise to two daughters lying side by side; so that we have $4a^a$ and $4a^p$, $4b^r$ and $4b^l$, $4c^a$ and $4c^p$, and $4d^r$ and $4d^l$, as we pass round the egg. Of these all but $4d^r$ and $4d^l$ enter into the formation of the gut wall: the last named will eventually give rise to those longitudinal streaks of cells known as the **mesodermic** or **germinal bands.** These bands will eventually become hollowed out to form the **coelom** or true body-cavity, the walls of which constitute the **adult mesoderm.**

The fifth quartette divides also at first evenly, 5a into $5a^1$ and $5a^2$, 5b into $5b^1$ and $5b^2$, 5c into $5c^1$ and $5c^2$, and 5d into $5d^1$ and $5d^2$. In quadrants A and B the division stops, but it goes on in quadrants

C and D; in these $5c^1$ and $5c^2$ divide into $5c^{11}$ and $5c^{12}$, and into $5c^{21}$ and $5c^{22}$, respectively; and the same thing happens to $5d^1$ and $5d^2$.

This greater growth in the hinder quadrants of the egg, which occurs both in the 3rd and 5th quartettes, has the effect of pressing the pairs of cells $5a^1$ and $5a^2$, and $5b^1$ and $5b^2$, out of their original arrangement of two lines converging to the lower vegetative pole of the egg, into a position of two lines parallel to one another, and they will eventually form the sides of the mid-gut (Fig. 106).

The residual macromeres 5A, 5B, 5C, and 5D, lastly divide, each into two equal daughters; 5C and 5A into anterior and posterior cells, 5B into right and left cells by radial divisions; but 5D divides into an inner and an outer cell $5D^1$ and $5D^2$, and the outer $5D^1$ divides again into $5D^{11}$ and $5D^{12}$, so that here, as in the fifth quartette, we have increased multiplication of cells in the hinder part of the egg.

These divisions complete all the divisions of cells which take place in the flattened plate-like blastula. We have, as we have already seen, 40 cells of the first quartette. Of the second we have 8 stomodeal, 4 larval mesoderm, and 26 cells forming the belt of flattened cells; *i.e.* 38 in all. Of the third quartette 8 enter into the formation of the stomodaeum, and 4 form larval mesoderm, 4 form larval kidneys, and 4 form ventral ectoderm ($3c^{2paa}$ and $3c^{pap}$, + $3d^{2paa}$ and $3d^{2pap}$, respectively), making 28 in all. The fourth quartette contains only 8 cells, the fifth 12, and there are 9 residual macromeres. So that the grand total of all the cells at this stage is, 40 + 38 + 28 + 8 + 12 + 9; *i.e.* 135 in all.

At this point of development invagination of the cells of the lower surface begins, and the blastula is converted into a gastrula which, in virtue of its apical plate and its four groups of prototrochal cells, may be already termed a **Trochophore.**

We shall now study how invagination is brought about. The nine residual macromeres form a plate at the vegetative pole. The two cells forming the centre of this plate, namely $5D^2$ and $5D^{12}$, rise upwards into the blastocoele, in consequence no doubt of altered chemical conditions here, that is, of altered **cytotaxis.** As the centre of the plate thus sinks in, two lateral ridges of cells become prominent and outline the edges of the indentation so formed; in a word they outline the **blastopore.** These lateral ridges are, on the right side, $5b^1$, $5b^2$, $4c^a$, $5c^{12}$, and $5c^{11}$; on the left side $5a^1$, $5a^2$, $4a^a$ $5d^{12}$ and $5d^{11}$.

The blastopore takes on the form of an oval opening, elongated in an antero-posterior direction. The front of the blastopore is formed by the cells $4b^r$ and $4b^l$, and the hinder end at this stage by the cells $4d^r$ and $4d^l$. The centre cell in each row ($4a^a$ and $4c^a$) approaches its opposite partner and so the ridge of the oval is converted into a figure of eight. These two latter cells finally meet one another and the oval opening is thus cut into two openings, the **primitive mouth** and the **primitive anus** respectively.

The primitive mouth persists, but the primitive anus is temporarily closed by the union of the four cells $5c^{12}$, $5d^{12}$, $5c^{11}$, and $5d^{1}$ respectively. At a later period of development, however, the **permanent anus** re-opens at the same spot, so that the temporary closure is an event of no importance.

We have thus the problem solved before our eyes how, out of a single primitive opening used both for injestion and egestion or defaecation, such as we find in Coelenterata and Platyhelminthes, separate openings for injestion and egestion were formed.

When the primitive anus is closed the blind end of the gut remains in close contact with the cells $4d^{r}$ and $4d^{l}$. Two outer columns of cells parallel to the first two are then formed. These consist on the right side of $2c^{222a}$, $2c^{222p}$, $3c^{2a2}$, and $3c^{2paa}$, and on the left side of $2a^{222a}$, $2a^{222p}$, $3d^{2a2}$, and $3d^{2paa}$. The hinder cells of these two outer ridges also meet; *i.e.* first $3c^{2a2}$ and $3d^{2a2}$, then $2a^{222p}$ and $3c^{222p}$, and lastly $3c^{2paa}$ and $3d^{2paa}$; but their front cells, $2c^{222a}$ and $2a^{222a}$, do not meet; they, as we have already seen, help to form the sides of the stomodaeum (Fig. 104, B).

As these outer columns of ectodermal cells meet, the endodermic pouch shrinks away from them and leaves a blastocoelic space between it and them; so that the process of closing the ventral wall of the gut is completed before the ventral ectoderm is complete, and thus, for a brief moment, the blastocoele is actually in open communication with the external world (Fig. 105, C).

The final closing of the outer part of the blastopore is effected by the rotation inwards and backwards of the cells $3c^{2paa}$ and $3d^{2paa}$. These cells rotate through an angle of 180°, and so come to lie actually within and behind the cells $3c^{2pap}$ and $3d^{2pap}$ (Fig. 104, D).

It is at this stage of development that the cells $3c^{2ppa}$ and $3d^{2ppa}$ wander like amoebocytes into the blastocoele and form the **solenocytes** of the two **archinephridia**. The pre-anal tuft of cilia, the **telotroch**, is formed by the cell $3d^{2paa}$. The lower lip of the large mouth is formed by the cells $3c^{1a}$, $3d^{1a}$, $3c^{2a1}$, $3d^{2a1}$, $3c^{2a2}$, $3d^{2a2}$, which swing through a right angle to occupy that position. The cells $3c^{1p}$, $3d^{1p}$, become elongated in an antero-posterior direction, acquire short cilia, and form the **metatroch**, *i.e.* the circular band of feebler cilia, which runs parallel to the prototroch behind the mouth (Fig. 104, D).

Turning our attention now to the second quartette in quadrant D, we find that the cell $2d^{222}$ wanders like an amoeba over the ventral surface of $4d^{r}$ and $4d^{l}$. Each of these cells has by this time budded off a small anterior cell, $4d^{r1}$ and $4d^{l1}$, which is the beginning of the adult mesoderm on each side.

We find now, when we look at the under side of the Trochophore behind the mouth, two large thin plate-like cells $3c^{2pap}$ and $3d^{2pap}$ in front. These constitute what Woltereck calls the **hyposphere**, or under surface of the almost spherical larva. To the sides of these, lie $3c^{2ppp}$ and $3d^{2ppp}$, the tubal cells of the archinephridia. Behind them are a group of three compact cells covering the adult mesoderm; and these cells which, for reasons to be explained later, we call the

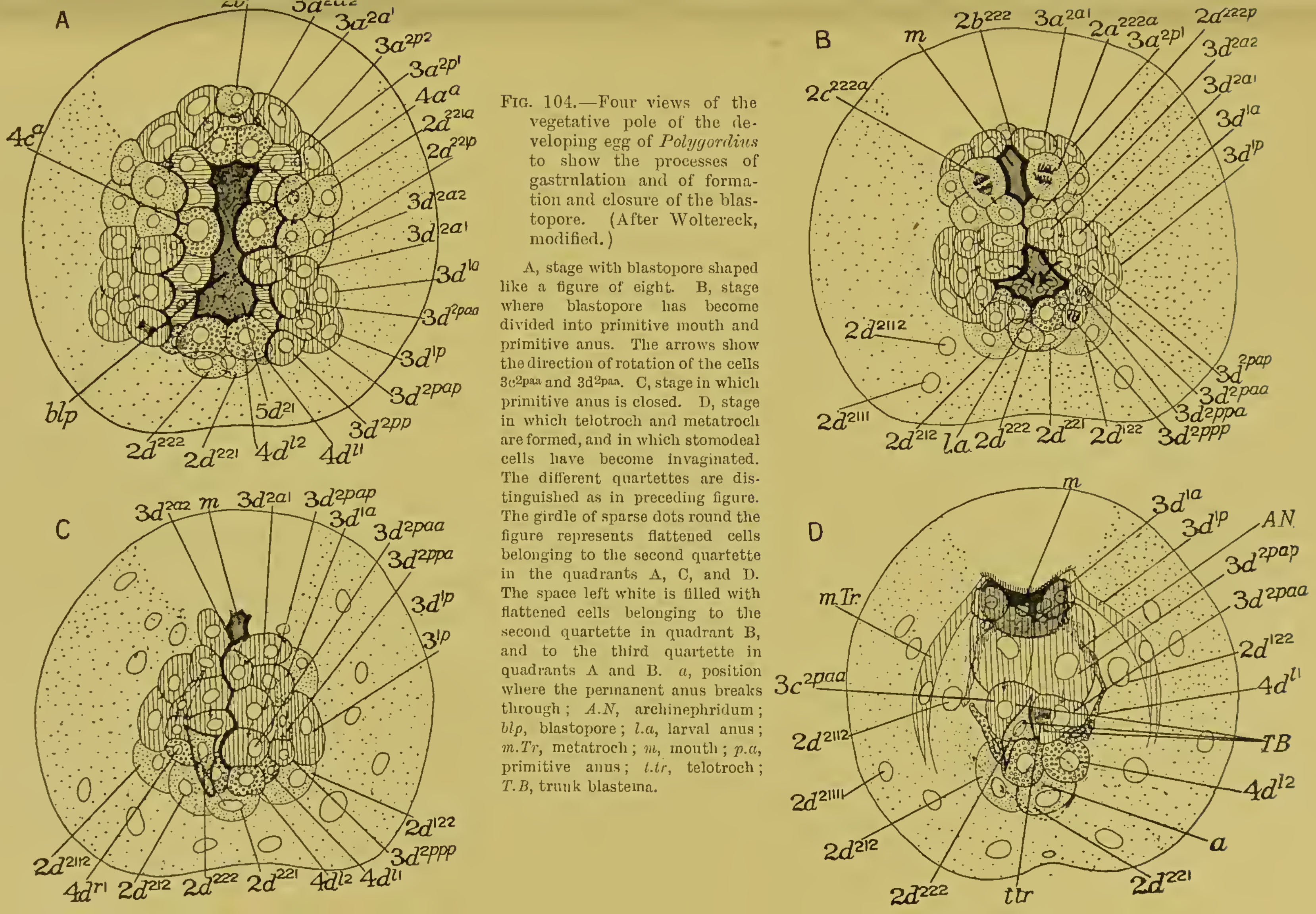


FIG. 104.—Four views of the vegetative pole of the developing egg of *Polygordius* to show the processes of gastrulation and of formation and closure of the blastopore. (After Woltereck, modified.)

A, stage with blastopore shaped like a figure of eight. B, stage where blastopore has become divided into primitive mouth and primitive anus. The arrows show the direction of rotation of the cells $3c^{2paa}$ and $3d^{2paa}$. C, stage in which primitive anus is closed. D, stage in which telotroch and metatroch are formed, and in which stomodeal cells have become invaginated. The different quartettes are distinguished as in preceding figure. The girdle of sparse dots round the figure represents flattened cells belonging to the second quartette in the quadrants A, C, and D. The space left white is filled with flattened cells belonging to the second quartette in quadrant B, and to the third quartette in quadrants A and B. *a*, position where the permanent anus breaks through; *A.N*, archinephridum; *blp*, blastopore; *l.a*, larval anus; *m.Tr*, metatroch; *m*, mouth; *p.a*, primitive anus; *t.tr*, telotroch; *T.B*, trunk blastema.

trunk blastema, are $3c^{2paa}$, $3d^{2paa}$, and $2d^{222}$. Outside and behind these are thin plate-like cells, belonging to the quadrant D of the second quartette, but immediately behind them lie the cells $2d^{212}$ and $2d^{221}$, which mark the point at which, about this time, the *anus is re-formed.*

Proceeding now to examine the cells which enter into the gut-wall, we find that the ventral surface of the gut is formed (1) by the union of the pair $4a^{a}$ and $4c^{a}$ which, as we have seen, by joining with one another, cut the original blastopore into two openings; and (2) by the union of the following pairs of cells belonging to the fifth quartette, viz. $5d^{12}$ and $5c^{12}$, $5d^{11}$ and $5c^{11}$, and at a slightly later period, the pair $5d^{21}$ and $5c^{21}$, which also unite with one another.

As the dorsal wall of the gut is invaginated the Trochophore swells out and becomes arched dorsally, recovering in this way from its flattened shape. The cells which form the dorsal wall, from being cubical become flattened and converted into thin arched shells of cytoplasm. The front wall of the mid-gut where it joins the stomodaeum is formed by the

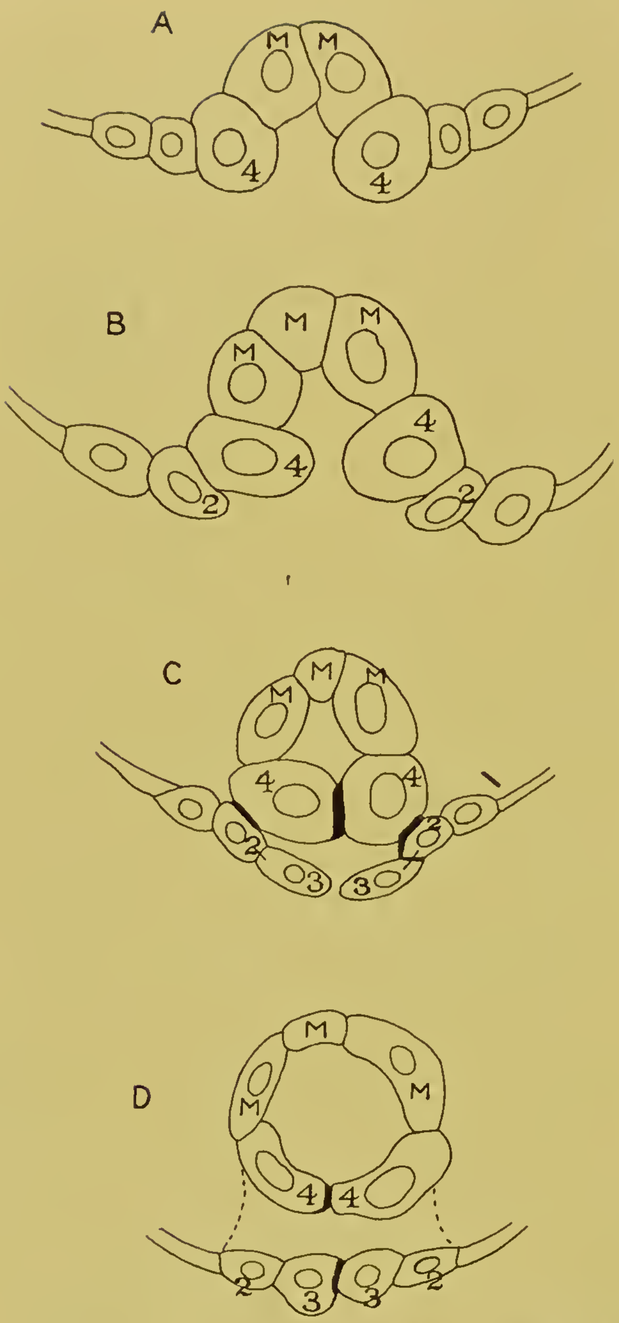


Fig. 105.—Four diagrammatic transverse sections of lower part of young Trochophore larva to show mode of closure of blastopore. (After Woltereck.)

A, gastrulation beginning; B, constriction of blastopore, the cells of fourth quartette approach one another; C, inner lips of blastopore closed; D, outer lips of blastopore closed. The figures refer to the quartettes to which the cells thus designated belong. *M*, residual macromeres.

cells $4b^l$ and $4b^r$, which, it will be remembered, were originally situated at the front rim of the long oval blastopore. Cells $5a^1$ and $5c^1$ also form part of the front wall of the mid-gut, being situated below $4b^l$ and $4b^r$. The lateral walls of the mid-gut are formed on the left by $5B^l$ above, by $5a^2$, in the middle, and $4a^a$ below; and on the right by the corresponding cells $5B^r$, $5c^2$, and $4c^a$.

The hinder wall of the mid-gut, where it joins the intestine, is formed on the left by $5A^a$, on the right by $5C^a$, and in the mid-dorsal line by $5D^2$. The **valve** which projects into the cavity of the gut and tends to separate the cavity of the mid-gut from that of the intestine, is formed by the following pairs of cells, one member of each pair being situated on the right side and the other on the left,—$4c^p$ and $4a^p$, $5B^p$ and $5C^p$, $5c^{22}$ and $5d^{22}$; and in addition by $5D^{12}$ in the mid-dorsal line.

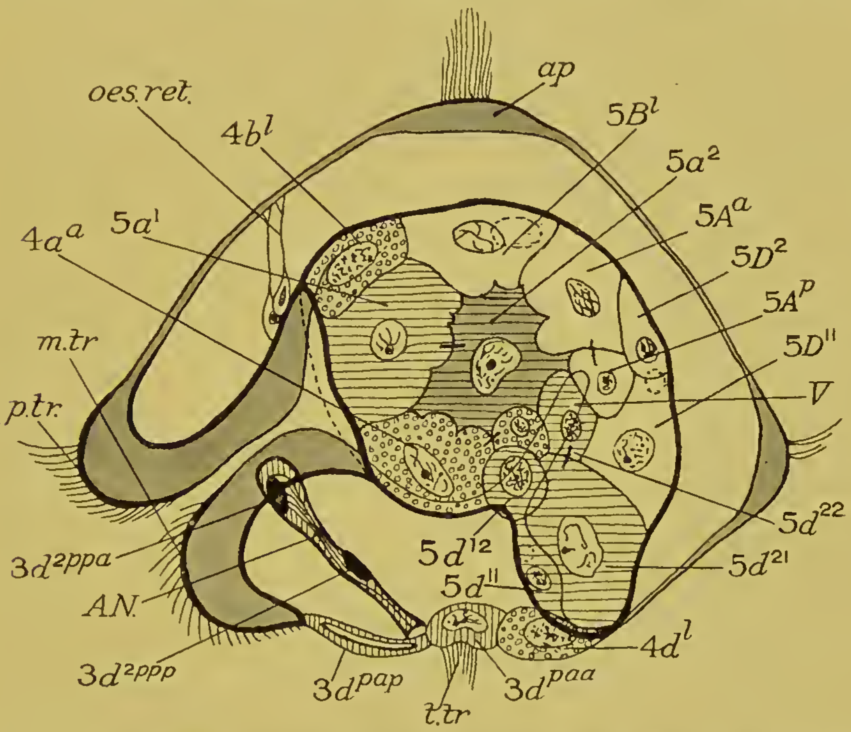


FIG. 106.—Optical sagittal section of young Trochophore after gastrulation is complete. (After Woltereck.)

Letters as in preceding figures. In addition, *V*, intestinal valve (this is represented in outline); *oes.ret*, larval retractor muscle of oesophagus.

The intestinal wall is formed by three pairs of cells, $5c^{12}$ $5c^{11}$ and $5c^{21}$ on the right, and $5d^{12}$, $5d^{11}$, and $5d^{21}$ on the left side, and by the single cell $5D^{11}$ in the mid-dorsal line. Its hinder wall is formed by $4d^{l2}$, $4d^{r2}$, which at this period form part of the gut wall but which later are the mother cells of the **adult mesoderm.** Between and slightly behind them, a little later, the **permanent anus** arises, and since they formed the hinder rim of the original blastopore and later of the primitive anus, it will be seen that the permanent anus corresponds to the hindermost piece of the primitive one.

The Trochophore is now complete and can begin to feed. It is the only larva in which the ancestry of every cell has been completely worked out, and hence its cell-lineage has been given with a completeness which it will not be necessary to repeat in the case of other forms. As now constituted it is very similar to the Pilidium, for the ventral surface is still almost flat and no trace of the projection which is to form the body of the future worm, has yet appeared. It is true that the Pilidium possesses no anus, but this, as we have seen, is also true of the young Trochophore.

The distinctive features of the Trochophore at this stage are the **metatroch**, the **archinephridia**, and the **telotroch**, all of which are wanting in the Pilidium.

FURTHER DEVELOPMENT OF POLYGORDIUS

There are two species or varieties of the European Polygordius, one found in the North Sea, *P. lacteus*, and one in the Mediterranean, *P. appendiculatus*. The segmentation of the egg and the early development up to the attainment of the Trochophore stage is strikingly similar in both varieties, but the later development, till the attainment of the adult form, is very different in the two cases. The adult worms according to Woltereck are practically indistinguishable from each other, so that we have here a curious instance of specific peculiarities being developed during the later larval history.

We shall describe the later development of the Neapolitan species first, as it is the simplest. The external features of this stage of development were exhaustively described by Fraipont (1887), but its true significance has only been made clear by Woltereck (1902 and 1905). In this species, after feeding has gone on for some time, a rapid period of cell division and growth sets in in the three cells forming the trunk blastema (these it will be remembered are $2d^{222}$ and $3c^{2paa}$ and $3d^{2paa}$); and in two cells, $4d^{rp}$ and $4d^{lp}$, the mother cells of the adult mesoderm which lie internal to the three cells just mentioned. These two latter cells had already, in the Trochophore, each budded off a small cell ($4d^{r1}$ and $4d^{l1}$) in front of them: this process is now repeated many times, and in this way two long strings of cells, the **mesodermic bands**, are formed. The original mother cells of the bands are termed **teloblasts.**

The multiplication of the cells of the trunk blastema furnishes new ectoderm to cover these bands, and in this way a post-trochal outgrowth of the body is formed. The intestine elongates at the same time by the growth and division of the cells forming its walls, for the anus is situated at the termination of this post-trochal projection.

By longitudinal divisions the mesodermic bands become several rows of cells thick, and a faint indication of the division of the post-trochal "body" into segments is now discernible. These segments are indicated in the ectoderm by faint transverse grooves parallel to one another; in the mesodermic bands by the appearance in each band of a set of cavities, which we may term somites, situated one behind the other, corresponding to the grooves in such a way that, in each segment, one pair of cavities is formed. The right and left somites in each segment rapidly expand and displace the blastocoele, and finally meet one another both above and below the intestine. Then, where their walls impinge on one another, absorption takes place, and so instead of two coelomic sacs one coelomic ring-shaped space is found in each somite.

Whilst these changes have been taking place the archinephridia have undergone further development, by the adhesion to them of further cells budded from the third quartette. The original pore cell divides and gives rise to a string of cells. The original flame cell, or solenocyte, persists, but the newly-added cells form additional solenocytes. The transformed nephridia are now known as the first pair of **protonephridia** (P.N^1, Fig. 108, A). Each consists of a tube which forks internally into two branches, and each branch terminates in two peculiar solenocytes. Each of these solenocytes consists of a head studded all over with blind cuticular tubes, each of which contains a flagellum (*sol*^{1}, Fig. 108, A). Behind this pair of protonephridia a second pair arises. Each of these consists of a tube opening by a pore on one side in the region of the first somite formed from the trunk blastema. The tube is terminated internally by solenocytes which are situated in the swollen body of the Trochophore, and which are derived from wandering cells, descendants probably of the third quartette. The tube itself owes its origin to a pore cell situated in the ectoderm of

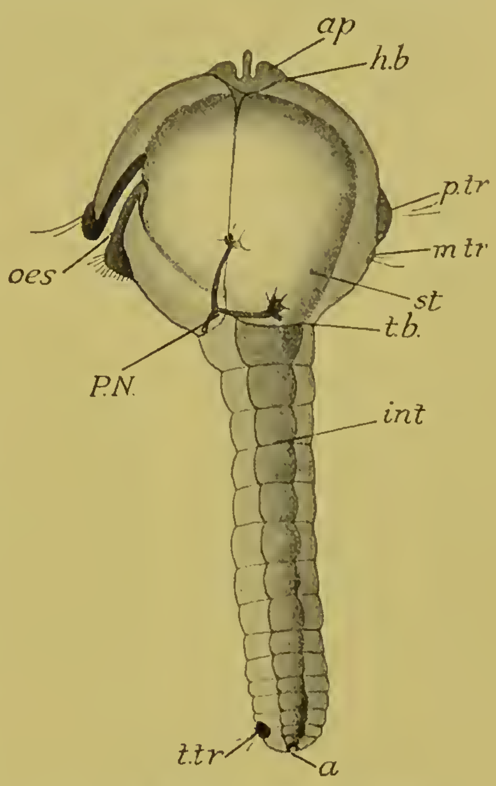


FIG. 107.—Later stage in the development of *Polygordius appendiculatus*, in which the "worm-body" is being formed by the growth of the trunk blastema. (After Woltereck.)

Letters as before. In addition, *a*, permanent anus; *h.b*, hind limit of head blastema; *int*, intestine; *P.N*, protonephridium; *oes*, oesophagus; *st*, stomach; *t.b*, front limit of trunk blastema.

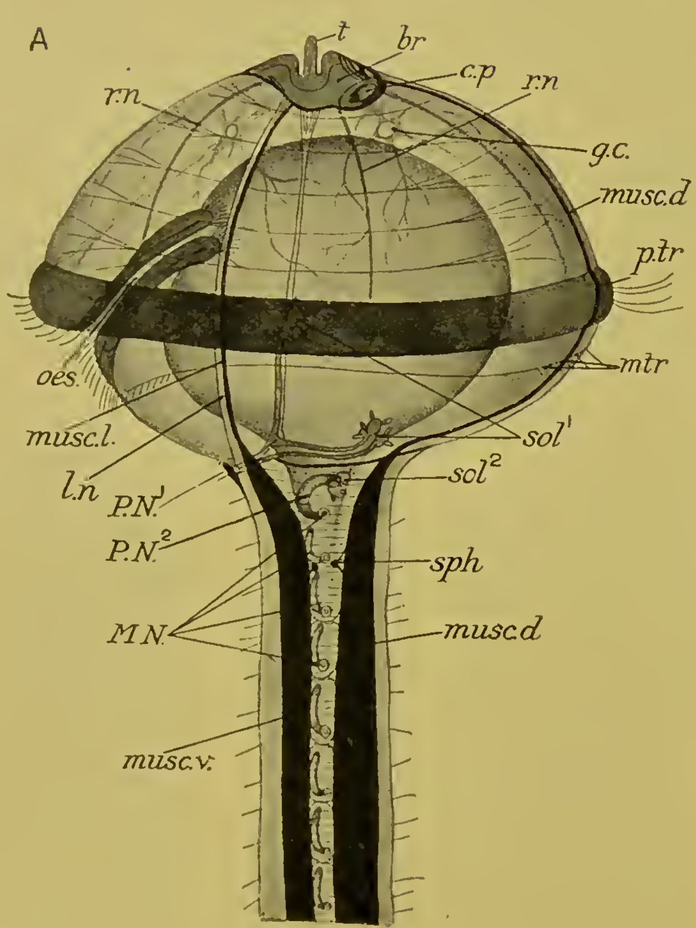
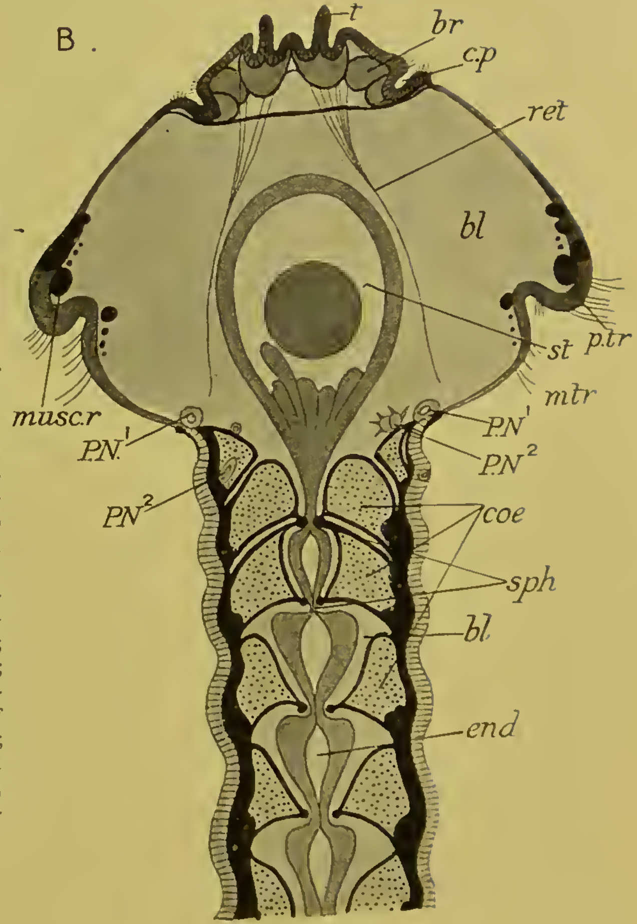


FIG. 108.—Longitudinal sections of stage represented in preceding figure to show details. (After Woltereck.)

A, optical sagittal section. B, frontal section. Letters as before. In addition, *br*, rudiment of brain; *bl*, blastocoele; *c.p*, ciliated pit; *coe*, coelomic cavities; *g.c*, ganglion cell of scattered nerve plexus; *l.n*, lateral nerve, the strongest radial nerve which forms part of the nerve collar of the adult worm; *musc.d*, dorsal sub-epithelial muscle passing into the dorsal longitudinal muscles of the trunk; *musc.l*, lateral sub-epithelial muscle passing into *musc.v*, ventral longitudinal muscles of the trunk; *musc.r*, ring muscle underlying the prototroch; *M.N*, rudiments of metanephridia; *r.n*, radial nerves; *P.N*¹, first pair of protonephridia; *P.N*², second pair of protonephridia; *sol*¹, solenocytes of the first pair of protonephridia; *sol*², solenocytes of the second pair of protonephridia; *sph*, sphincter of gut; *ret*, main retractor muscles of the apical plate.



the trunk blastema; this cell buds so as to form a string of cells, which become hollowed out so as to constitute a tube.

Further cell multiplication has also been proceeding in the region of the rosette or "Annelidan cross," around the apical plate, and this region of new-formed cells is termed the **head blastema.** On each side, just as in *Planocera* (see page 110), cells are budded inwards into the blastocoele and form the rudiments of the **larval cerebral ganglion.** Other cells of the larval mesenchyme form muscular strands which reach from the apical plate to the sides of the oesophagus and to the ventral ectoderm in front.

As the post-trochal outgrowth of the body increases in length its tissues begin to undergo histological differentiation, but unfortunately the details of this have not yet been worked out in *Polygordius.* As more and more somites are formed we can make out, in the most anterior and advanced somites, the **longitudinal muscles.** These appear to be laid down as hard refractive fibrillae in the basal portions of the cells forming the outer wall of the coelom; the **visceral muscles** also make their appearance as similar fibrillae in the basal parts of the cells forming the inner wall of the coelom.

The origin of the posterior or permanent nephridia, the **metanephridia,** as we may term them, has not been fully worked out. Woltereck asserts that they arise as strings of cells, growing from "pore-cells" situated in the ectoderm of the trunk blastema. At an early stage of their development they appear as small packets of cells lying in the outer wall of the coelom, where the insertion of the oblique septum separates dorsal and ventral bundles of longitudinal muscles. In the adult, as is well known, the inner ends of these nephridia communicate with the coelom by ciliated funnels. How these are formed has not been made out in *Polygordius.*

The origin of nephridia in Annelida generally has been the subject of much dispute, but a great deal of light has been thrown on this subject by the results of investigations on the development of the Oligochaete worm *Criodrilus,* details of which will be given later.

The head blastema undergoes important changes as the larva grows older. We have already seen that underneath the apical tuft of cilia there is formed a mass of nerve cells and nerve fibres, which constitute the larval cerebral ganglion. From this ganglion there proceeds eight radial nerves which go to the cells forming the prototroch. Two of these, termed **lateral nerves,** are stronger than the rest; these cross the prototroch and join the ectoderm cells which will give rise to the **ventral nerve cord** of the adult. They constitute the **nerve collar** of the adult. Of the six other radial nerves, two are situated on the anterior side of the larva, whilst four are on the posterior side.

Besides these radial nerves there is a network of ganglion cells and fibres underlying the ectoderm of the upper half of the larva, and, though in a much sparser condition, beneath a good deal of the ectoderm of the lower half. There is also a ring of nerve fibres underlying the prototroch, and another underlying the metatroch. Apparently the radial nerves do not communicate with the plexus, and in this respect the Trochophore resembles a Ctenophore, for there is in the adult Ctenophore a plexus of nerve fibres under the ectoderm which is quite distinct from the radial bands which underlie the "ribs."

As the larva grows the apical ciliated cells fall off and the spot where they existed becomes a pit, soon overgrown from the sides by thickenings of the ectoderm. These lateral masses (probably derived from the Annelidan cross) form short thick pits from whose bases the **head tentacles** arise (*t*, Fig. 108). The ganglion, however, persists for some time, but it is soon overshadowed by two lateral masses of nuclei which constitute the rudiment of the paired supra-oesophageal ganglion of the adult. These masses lie beneath and to the outer sides of the rudiments of the tentacles; they probably arise from the inner parts of the lateral arms of the "Molluscan" cross; they enter into connection with the two lateral nerves which ultimately form the nerve collar of the adult. Behind the tentacle bases there appear two shallow grooves: these acquire, later, stiff cilia and become the **ciliated pits** (*c.p*, Fig. 108) of the adult. At the extreme outer edges of the head blastema there appear two small pits lined by pigmented cells. These are the **eyes** of the larva.

Underneath the lateral nerves the **lateral muscles** are formed later by the accumulation of mesenchyme cells. These connect with the trunk blastema and persist, though they do not become functional until the metamorphosis. A **mid-dorsal muscle** is formed in the same way and is not functional during the larval period.

These sub-epithelial muscles which are taken over into the adult, are not to be confounded with purely larval muscles, mentioned above, which traverse the blastocoele. These transitory larval muscles are, (1) a pair of **oesophageal retractors** running from the head blastema to the skin just above the mouth (*oes.ret*, Fig. 106); (2) a pair of **main retractors** running from the head blastema underneath the origin of the tentacles to the trunk blastema (*ret*, Fig. 108, B); (3) a pair of **dorsal "elevators"** (*elv*, Fig. 112) arising from the dorsal surface half-way between the apical plate and the prototroch ring, and running downward to join the trunk blastema; (4) a **plexus** of muscle fibres underlying the skin everywhere and crossing each other in various directions; and (5) two powerful **ring muscles**, one underlying the prototroch and one the metatroch.

Before the growth of the post-oral segmented region is quite complete, the intermediate girdle cells intervening between the region of the prototroch and the "head blastema," gradually degenerate and disappear. They pass from a flattened to a columnar form, their nuclei are dissolved, they assume a glassy appearance and then are cut off either internally, when their remains are devoured by amoebocytes, or externally; and so the head blastema comes into contact with the prototroch ring on its upper margin. A few of these intermediate cells, however, just at the margin of the head blastema, become transformed into gland cells (*gl*, Fig. 109), which are almost certainly of an excretory nature.

A similar contact takes place between the lower end of the prototrochal ring and the front end of the new ectoderm, forming the trunk blastema (arising chiefly from the descendants of the cell $2d^{222}$); so that the cells forming the hyposphere (descendants of $3c^{2pap}$ and $3d^{2pap}$) have also been absorbed.

The larval oesophagus gradually loses its cellular walls, which are replaced by new cells budded from two lateral pockets. These pockets may be compared to the "**imaginal discs**" of insects (see p. 267). The renewed oesophagus is delimited from the stomach by a ring of ciliated cells. From the walls of the stomach, cells are given off into its cavity and degenerate, and in this way the globular stomach slowly decreases in size. The boundary separating stomach from intestine is, of course, marked by a projecting valve. This is armed with stiff cilia so as to constitute a **ciliated filter** (*c.f*, Fig. 109).

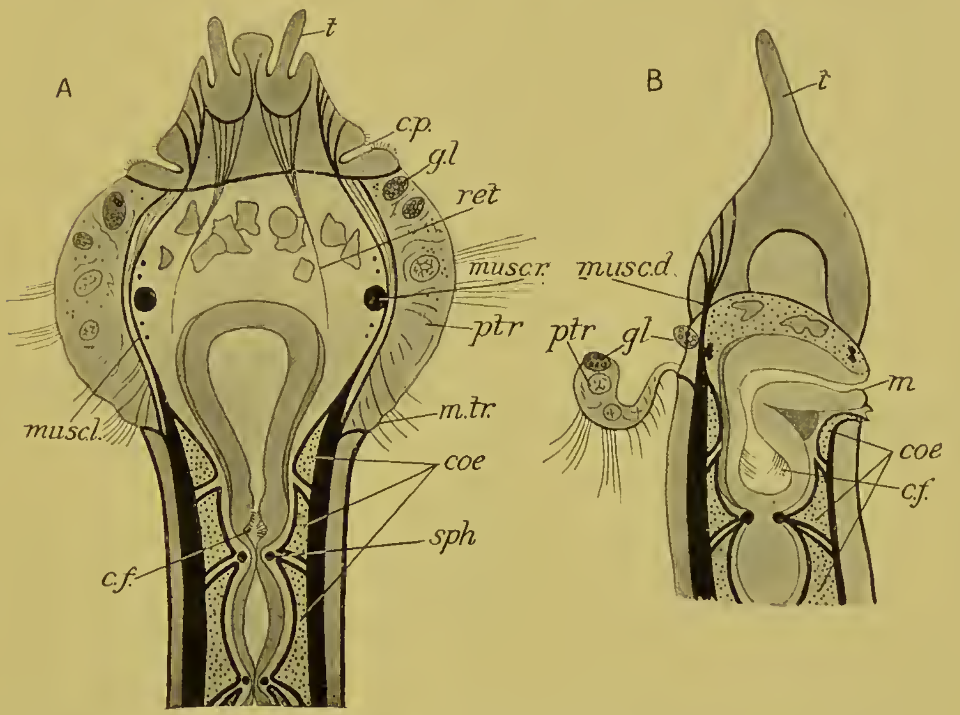


FIG. 109.—Two sections of the anterior part of a larva of *Polygordius neapolitanus* to show the changes supervening on metamorphosis. (After Woltereck.)

A, frontal section just before metamorphosis. B, sagittal section just after metamorphosis. Letters as before. In addition, *c.f*, ciliated filter; *gl*, excretory gland cells. The coelomic cavities of the somites are dotted but the blastocoele has a pale uniform tint.

When the growth of the post-oral region has reached its full extent cataclysmal changes take place. The circular muscles underlying the prototroch contract so vigorously as to cut the skin uniting the edges of the head and trunk blastemas and of the prototroch. The oesophageal and main retractors, and the three sub-epithelial muscles, contract and pull the head blastema down until it is in contact with the upper end of the trunk blastema. The prototroch is cast off as a crumpled collar.

The globular stomach is to a large extent dragged back into the trunk by the peristaltic swallowing action of the visceral muscles, which are not closely adherent to the gut wall. The sphincter separating the end-gut from the stomach closes; the remains of the stomach are then broken up and devoured by phagocytes and the renewed oesophagus comes into contact with the end-gut. Thus the intestine is again completed.

The retractors of the oesophagus and the main retractor and elevator muscles are broken through by the intense contraction, and absorbed, but the sub-epithelial muscles persist and become continuous with the ventral and dorsal longitudinal musculature of the adult.

The first pair of protonephridia break up and disappear, and so do the heads of the second pair of nephridia, but the U-shaped tube of each of these persists as a nephridium of the first segment or **peristomium.**

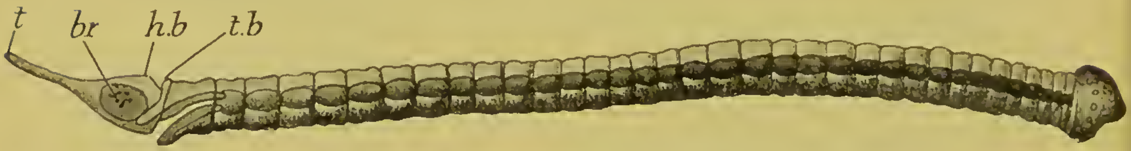


Fig. 110.—*Polygordius neapolitanus* immediately after metamorphosis. (After Woltereck.)

Letters as before. In addition, *h.b*, hinder limit of head blastema; *t.b*, frontal limit of trunk blastema.

The two coelomic sacs which are contained in the peristomial segment, diverge in front, and between their dorsal ends a section of the blastocoele projects backwards and becomes continuous with the dorsal and ventral **blood-vessels**; the vessels themselves indeed are but the spaces between right and left sacs in successive segments. The lining of these blood-vessels is provided by free wandering amoebocytes which apply themselves to their walls (*mes*, Fig. 111).

Immediately after the metamorphosis brain and pharynx are in close contact, and the blastocoele in this neighbourhood is squeezed out, but later a space between them again develops, which we may term the **schizocoele** (*schiz*, Fig. 111). The pharynx becomes surrounded by a thick mantle of cells derived from the descendants of $3a^2$ and $3b^2$, and this mantle becomes split into an inner and outer layer. From the latter are derived **radiating muscles**; these are inserted at one end in the ectoderm, while in the other direction they traverse the space between inner and outer layers and insert themselves in the oesophageal cells. The inner layer become nerve cells, whence nerve fibres run along the pharynx in a longitudinal direction (*oes.m*, Fig. 111, A).

When the animal has stripped off its prototroch it sinks to the bottom: the eye-pits become functional, but their principal use is to avoid the light. When eventually the adult adopts a burrowing life the eyes generally degenerate.

In the North Sea species, *P. lacteus*, the later development is somewhat different from that which we have just described. Thus, a ring-shaped invagination of the thin ventral surface of the larva is formed, giving rise to a circular fold, which we shall term the **amniotic fold**, surrounding the trunk blastema; and the space between this fold and the ectoderm covering the trunk blastema—in a word, the cavity of the invagination—grows forwards and upwards in the form of four pockets, a dorsal, a ventral, and two lateral.

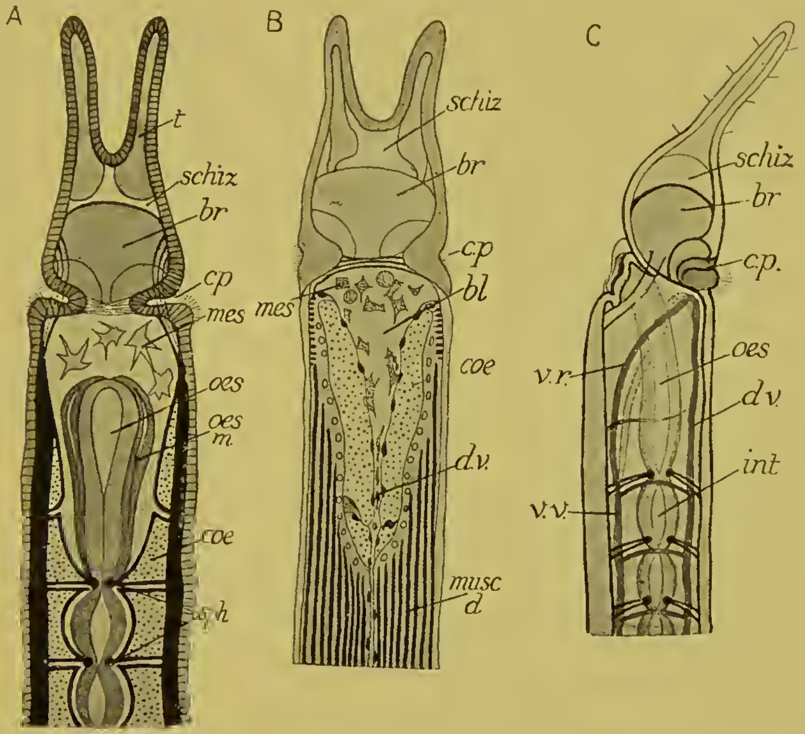


FIG. 111.—Longitudinal sections of anterior portion of *Polygordius neapolitanus* immediately after metamorphosis, to show details. (After Woltereck.)

A, frontal section at level of oesophagus and intestine. B, frontal section dorsal to intestine. C, sagittal section. Letters as before. In addition, *bl*, dorsal blood-vessel; *oes.m*, mantle of ectoderm cells surrounding oesophagus; *schiz*, schizocoelic space in front of brain; *b.r*, blood ring; *v.l*, ventral blood-vessel; *mes*, wandering cells in the blastocoele.

When further growth and cell division begin in the trunk blastema the new post-trochal body thus formed is folded up within the cavity covered by the circular fold just mentioned, and only at the moment of metamorphosis is the body straightened and thrust forth as the segmented trunk of the adult worm. Previous to this period it is held in its compressed and crumpled condition, along its surface to its posterior end, by extensions of the main blastocoelic retractor and elevator muscles. When these break through during the intense contraction which accompanies metamorphosis, the straightening process begins.

A further difference between the two species of *Polygordius* is found in the condition of the nephridia. In *P. lacteus* the archinephridia remain small and unbranched, and the only change which they undergo in becoming converted into the first pair of protonephridia is to become covered with numerous cuticular tubes—each containing a flagellum—so as to resemble, in fact, *one* of the solenocytes of the first pair of protonephridia of *Polygordius appendiculatus*. The second pair of protonephridia take the form of long tubes studded all over with solenocytes, which are directed dorso-ventrally, parallel with the prototroch, and which open dorsally at the beginning of the post-trochal region.

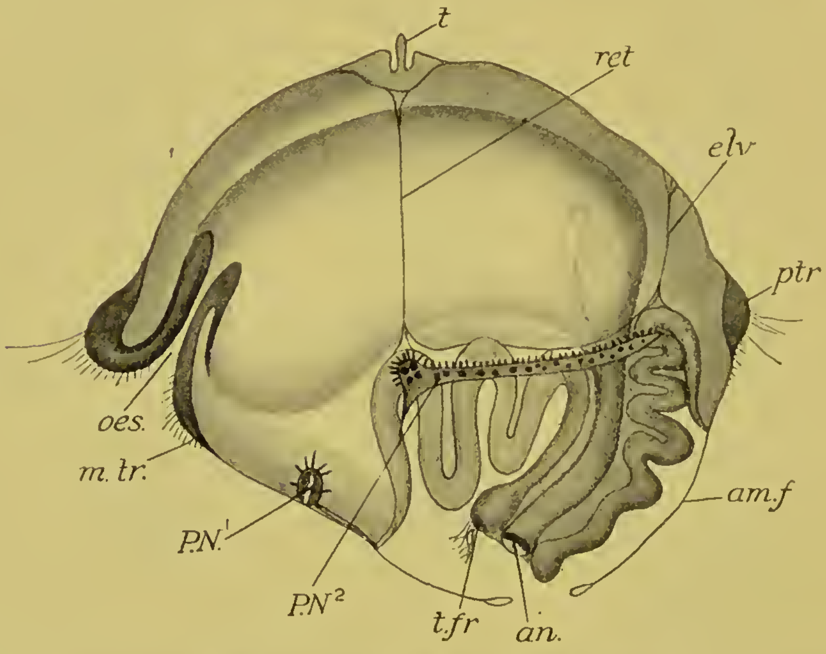


FIG. 112.—Late larva of *Polygordius lacteus* in optical frontal section. (After Woltereck.) Letters as before. In addition, *am.f*, amniotic fold; *elv*, larval elevator muscle; *t.fr*, telotroch.

It is obvious that the development of *P. lacteus* as compared with that of *P. appendiculatus* is shortened and modified. In some points indeed it reminds us of the development and metamorphosis of the Pilidium larva of the Nemertinea. Thus we could compare the four amniotic invaginations of the Pilidium larva to the four pockets which arise from the circular groove in the Trochophore, since in both cases the adult body is formed from their inner walls; but the

Pilidium differs radically from the Trochophore in the fact that its apical plate is cut off with the rest of the larval skin, whereas in the Trochophore larva it persists and forms part of the covering of the adult head.

REMAINING ANNELIDA

A great number of other species of Annelida have been worked at, chiefly Polychaeta, but in no single case with anything like the thoroughness with which Woltereck has worked out *Polygordius*. The Oligochaeta and Hirudinea have no free-swimming larvae. Their eggs are laid enclosed in cocoons along with a milky nutritive secretion, and within these they pursue their development until they attain the adult form; so that, as one might expect, the development is profoundly modified as compared with the Trochophore of *Polygordius*; nevertheless in broad outlines the same general course of development can be discovered.

POLYCHAETA

Turning our attention now to the Polychaeta we find that the first important point to be borne in mind is that a true Trochophore larva is found in very few cases. In most cases the rapid development of the ectodermal descendants of $2d^2$, which in *Polygordius* marks the conclusion of the Trochophore stage, begins long before the embryo escapes from the egg membrane; so that when it does escape it has the form of a post-trochophoral stage with several somites already developed (Fig. 116).

In a word the development of such worms is, as compared with that of *Polygordius*, "telescoped." This is true of *Nereis* among Nereidiformia, *Capitella* among Capitelliformia, *Arenicola* among Scoleciformia, and *Amphitrite* among Terebelliformia. In other cases, such as *Sternaspis*, the endoderm consists of large yolky cells, and neither mouth nor anus is developed when the larva begins its free existence.

It was formerly customary to classify Annelidan larvae by their ciliated bands. Such classification is obsolete, but we shall give the significance of the terms as they are still used by some. **Atrochal** larvae are those in which there is an apical tuft of cilia and a general ciliated covering, but no prototroch. Such is *Sternaspis*; of course they are degenerate forms. **Monotrochal** larvae with prototroch only, are the early stages in the typical development of the Trochophore; they become later **telotrochal**—by the development of the telotroch as described in *Polygordius*. **Mesotrochal** larvae, such as that of *Capitella*, are forms where the **metatroch** only is developed, the prototroch being undeveloped. **Polytrochal** larvae are really post-trochophoral stages in which accessory hoops of cilia are developed on the worm's body to assist in locomotion (Fig. 116).

As we have already hinted, however, in many of the Sabelliformia there is a development closely resembling that of *Polygordius*, and the animal begins its larval life as a true Trochophore. In the genus *Eupomatus*, according to Shearer (1911), the blastopore divides into primitive mouth and primitive anus—as in *Polygordius* the mouth opening persists but the primitive anus is closed ; later the permanent anus is formed just where the primitive anus disappeared. In this form, when the Trochophore has begun to swim about, there is as yet no trace of adult mesoderm; the mother cells of this layer $4d^r$ and $4d^l$ are still situated in the wall of the intestine, and only when the larva has been leading a free-swimming life for a day or two do these cells emerge from the gut wall and begin to found the mesoderm bands. Further, in *Eupomatus*, the two archinephridia, which are formed exactly as in *Polygordius* by the migration inwards of certain cells of the third quartette and which develop so as to form the only pair of protonephridia present in this larva, persist in the adult, and here constitute the two large effective nephridia which serve as principal excretory organs; they are situated in the prostomium and open by a dorsal pore. According to Shearer they open near the anus in the larva, but their opening becomes shifted by the growth of the "worm-body." The Trochophore of *Eupomatus* is further remarkable for developing a large clear vacuole, the so-called **anal vesicle** (*an.v*, Figs. 113 and 114), in the cells which support the telotroch.

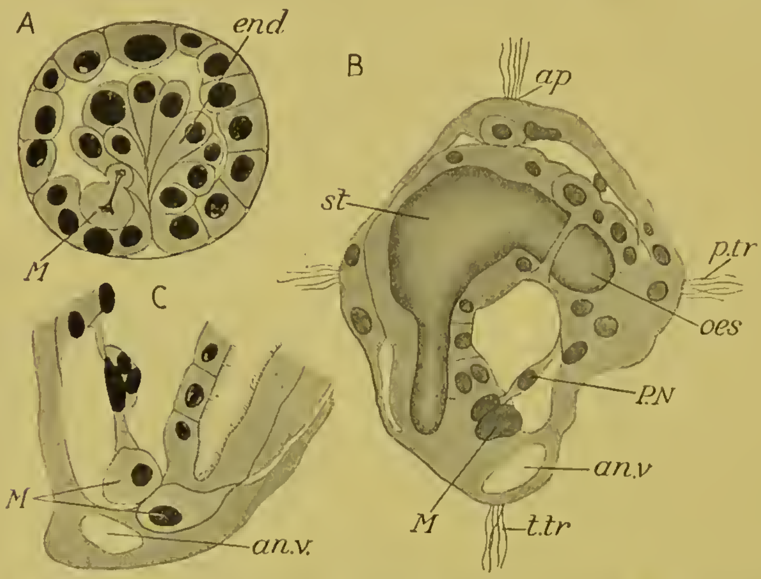


FIG. 113.—Figure illustrating the origin of the mother cells of the adult mesoderm in *Eupomatus*. (After Shearer.)

A, section of young gastrula showing the inward migration of endoderm cells and the formation of the mother cell of the mesoderm by the division of one of them. B, sagittal section of Trochophore larva three days old. C, sagittal section of hinder end of Trochophore larva, much enlarged. *an.v*, anal vesicle ; *ap*, apical plate ; *end*, endoderm ; *M*, mother cell of mesoderm lying in endoderm ; *p.tr*, prototroch ; *P.N*, protonephridium ; *st*, stomach ; *t.tr*, telotroch.

In the two following points, namely, the primitive situation of the mother cells of the mesoderm in the wall of the gut, and the persistence of the protonephridia, *Eupomatus* can fairly claim to show, on the whole, a more primitive development than even *Polygordius*. A detailed study of the development of this form, or of that of the allied genus *Pomatoceros*, is greatly to be desired.

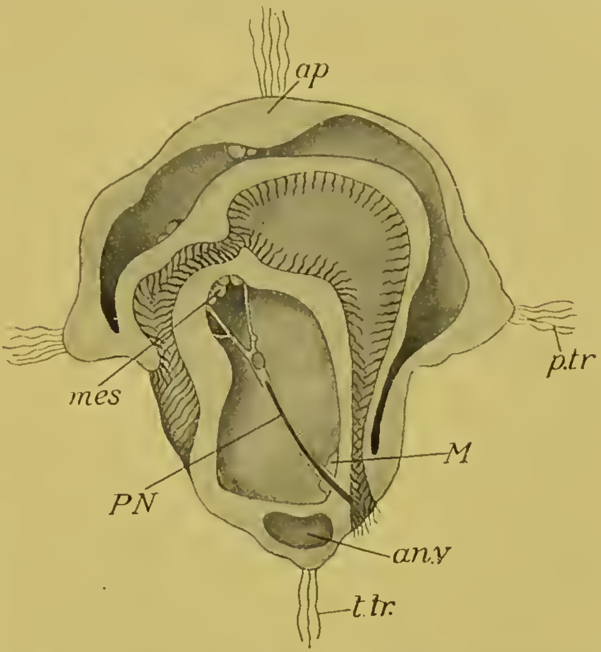


Fig. 114.—Diagrammatic saggital section of fully-grown Trochophore larva of *Eupomatus* to show the relative position of the protonephridia and the coelomic rudiment. (After Shearer.)

Letters as before. *mes*, larval mesoderm, consisting of ectoderm cells budded inwards from the second quartette.

Turning now to *Nereis* as an example of the "telescoped" form of development, described in detail by Wilson (1892), the first difference that strikes us as compared with *Polygordius* is that the macromere D is, from the first, distinguishable from the other three by its greater size. The first quartette of micromeres are, however, equal in size and their development is, in broad terms, the same as in *Polygordius*. In each quadrant, however, one of the trochoblasts does not develop cilia, but joins the intermediate girdle cells to form part of the covering of the upper hemisphere.

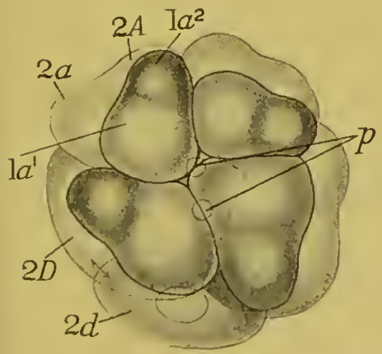


Fig. 115.—Stage in the segmentation of the egg of *Nereis limbata* viewed from above, showing a laeotropic spiral cleavage of the egg. (After Wilson.)

p, polar bodies.

When, however, the second quartette of micromeres are given off, the posterior one, 2d, called by Wilson "the **first somatoblast,**" is very much larger than its sisters, and after a few preliminary divisions proceeds to bud off that ventral plate of ectoderm cells which covers the post-trochal "body" of the worm (Fig. 115).

The third quartette is normal, but in the case of the fourth quartette, 4d, termed by Wilson the **second somatoblast,** is formed some considerable time before its sisters, and at once divides into right and left daughters, and these begin to found the mesodermic bands. Wilson has recently pointed out, however (1898), that 4d is sufficiently reminiscent of its endodermic origin to contribute six to ten small cells to the formation of the intestinal wall.

When the larva of *Nereis* is fully developed, *i.e.* when the alimentary canal has become functional, it is not only provided with a post-trochal worm-body but this body shows the rudiments of no less than three pairs of parapodia, and this seems to be a general feature amongst many Polychaeta. Further, behind each pair of parapodia is an accessory ring of cilia, so that in *Nereis* we have a typical example of a Polytrochal larva. In the apical region there is to be seen a row of five gland cells—these seem to be homologous with the gland cells in the upper hemisphere of the Trochophore of Polygordia.

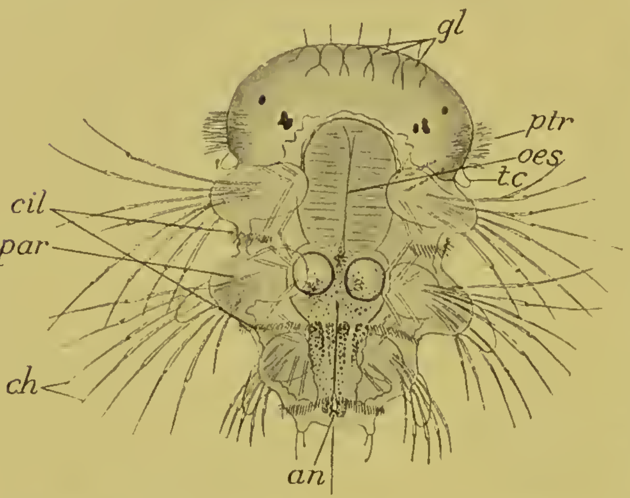


Fig. 116.—The free-swimming larva of *Nereis limbata* three days old. A typical "Polytrochal" larva. (After Wilson.)

an, anus; *ch*, chaetae; *cil*, additional ciliated rings; *gl*, gland cells; *par*, parapodia; *t.c*, tentacular arms.

The development of the genus *Capitella* has been worked out in great detail by Eisig (1900). His results are chiefly remarkable for his assertion that, in this form, the mother cells of the adult mesoderm arise from the third and not the fourth quartette of micromeres. We are, it seems to us, justified in questioning this result, since it would place *Capitella* in opposition to every other Annelidan type which has been studied.

But Eisig records a feature from the post-trochophoral stage which is not recorded of *Polygordius*, but which we have other reasons for regarding as of an extremely primitive character, and this is a ciliated groove stretching from mouth to anus. The same feature recurs in Trochophore larvae belonging to quite different groups of the animal kingdom.

OLIGOCHAETA

In the Oligochaeta the prototroch is never developed at all: the embryo develops into a blastula, which is converted into a gastrula by invagination or by epibole. The mesoderm arises as a pole cell from the lip of the blastopore which divides into two, and these two halves proceed immediately to give rise to the mesodermic bands. The blastopore persists as the mouth, and the embryonic gut becomes swollen and globular owing to the ingestion of the nutritive material, the albumen, with which the embryo is surrounded.

The question of the origin of the **nephridia** in Annelida is a subject which has been much worked at in Oligochaeta where, owing to the fact that the eggs are contained in quantities in a cocoon, all stages of the embryo up to those that show all the adult features, can be obtained in quantity. The original conception of a nephridium as a tube connecting the coelom with the exterior, governed the early investigations into the subject. Bergh (1899) and Bürger (1902) asserted, that in Oligochaeta the nephridium arose as a growth of the septal wall of the coelom, that it gave rise to a chain of cells projecting backwards, which eventually fused with the ectoderm and then became hollowed out, so that the whole nephridium is to be looked on as a "tail" of the coelom. Moreover since the first trace of a cavity appears in the funnel region and is a prolongation of the body-cavity, the cavity of the nephridium might be said to be part of the coelom.

This view was attacked by Goodrich (1897–1898), who showed that in certain Polychaeta (cf. *Nepthys*) the nephridia do not open into the coelom at all but terminate internally in a bunch of solenocytes which project into the coelom. He regarded the nephridium as essentially an ectodermic structure, and as comparable with the excretory tube of a Nemertine or of a Platyhelminth. He believed that in a great many Annelida these blind nephridia had secondarily acquired openings into the coelom, but that on the other hand there were other so-called nephridia, cf. the large anterior nephridia of Terebellidae and Arenicolidae and the nephridia of Mollusca and *Peripatus*, which did actually develop as outgrowths from the coelom, and which in consequence he termed **coelomiducts.**

Goodrich regards the excretory organs of Oligochaeta as "true" nephridia not coelomiducts, *i.e.* as tubes originally blind which have acquired secondary communications with the coelom, and he pointed to the coexistence of the genital duct (which is a wide short coelomiduct) and the nephridium in the same somite, in *Lumbricus*, as evidence that the two structures cannot be homologous with one another. Evidently the question as to which category these nephridia belong can only be answered by renewed and exhaustive research into the mode of their development.

This has been done by Staff (1910) in the case of the worm *Criodrilus lacuum*. A word or two on Staff's method's may be in place here. *Criodrilus lacuum* is a worm which inhabits swamps on the banks of the Danube. In May and June when the swamps are nearly dry its cocoons are found attached to the stalks of the grasses growing in the swamp. These cocoons are collected, and they are then pressed at one end and the contained embroyos are thus squeezed out. They are preserved in "Eisig's mixture." Then they are examined under a strong dissecting microscope, and slit open along the back with a fine needle. In this way the ball of albuminous material filling the gut can be removed, and with care the endoderm itself can be removed. What is left consists of the mesodermic bands and the underlying ectoderm with its products. This can either be cut into sections or flattened out and mounted whole. Since development progresses from front to back, and since in one and the same specimen well-developed cœlomic cavities and nephridia may be found in front whilst only mother cells of the mesoderm and undifferentiated mesodermic bands are found behind, the whole development of many organs can be elucidated by examining a few embryos of suitable age. Staff found that, in the case of this species, the mother cells of the nephridia appear *in the ectoderm* at the hinder region of the embryo, and here act as **teloblasts,** giving rise to strings of cells by continuous budding off of smaller cells in front of them, like the mesodermic teloblasts situated internally to them. There are on each side four rows of such ectodermal teloblasts, and the rows of cells to which they give rise become wedged in between the ectoderm and the coelomic mesoderm. The two most ventral rows give rise to the **ventral nerve cord,** the row lying immediately outside these on each side to the **nephridia,** and the two uppermost rows on each side to the **external circular muscles.** The last-named teloblasts are consequently termed **myoblasts.**

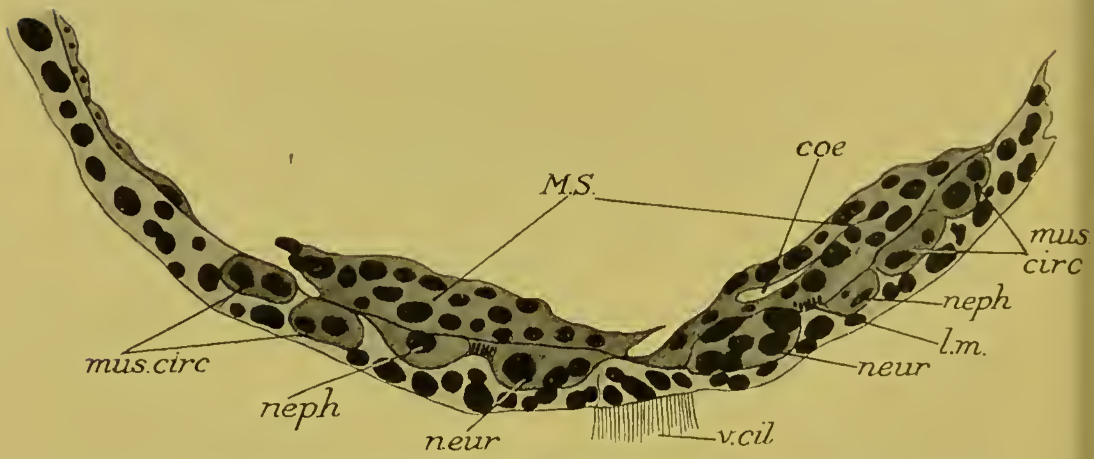


FIG. 117.—Transverse section through the ventral part of an embryo of *Criodrilus lacuum.* (After Staff.)

coe, coelomic cavity; *l.m*, first formed fibrils of the longitudinal muscles; *M.S*, somite; *mus.circ*, ectodermic cell group destined to give rise to the outer circular muscles; *neph*, ectodermic cell group destined to give rise to the nephridia; *neur*, ectodermic cell group destined to give rise to the ventral nerve cord; *v.cil*, ventral ciliated patch of ectoderm.

The longitudinal muscles and the **visceral muscles** of *Criodrilus* and Oligochaeta generally, like those of *Polygordius*, are derived from the cells of the wall of the coelom. At a later period the strings of cells destined to give rise to the nephridia are broken into groups, and one group is pushed into each septum which divides one coelomic sac from another. Here each group grows and gives rise to a chain of cells, and this cell chain becomes hollowed out and forms a tube. Its most internal cell projects into the coelomic cavity between the coelomic cells forming one side of the septum, and forms the greater part of the coelomic funnel of the nephridium. The lower lip of the funnel is constituted by one huge cell belonging to the coelomic wall. This cell (*f.c*, Fig. 118) only divides once and, as is well known, the ventral lip of the nephridial funnel in the adult worm is quite different from the dorsal lip, and consists of only two cells. The result of this investigation is therefore to uphold Goodrich's view.

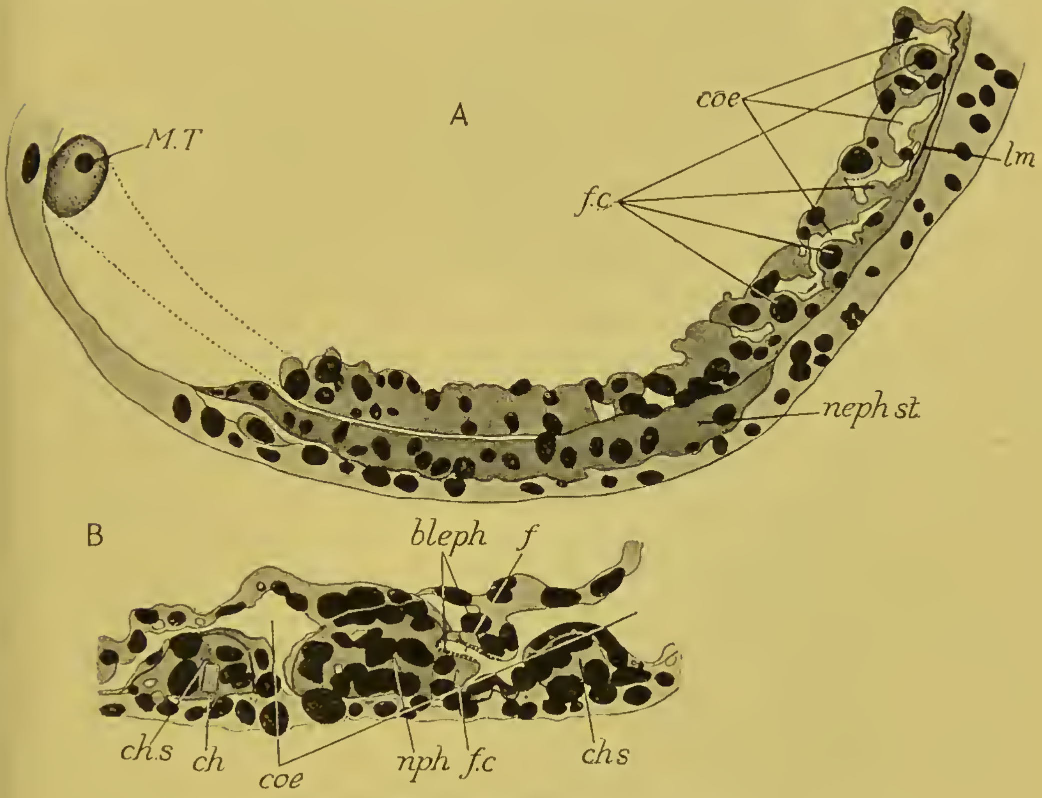


FIG. 118.—Two longitudinal sections through the ventral portion of an embryo of *Criodrilus lacuum*. (After Staff.)

A, section through a younger embryo in which the nephridial string is not yet broken into cell-groups destined to give rise to the nephridia. B, section through an older embryo in which the nephridial string is broken into groups. Letters as before. In addition, *bleph*, blepharoblasts, *i.e.* basal granules of cilia in the funnel of nephridium; *ch*, rudiment of chaeta; *ch.s*, group of ectoderm cells destined to form the chaeta sac; *f*, funnel of nephridium; *f.c*, funnel cell, *i.e.* the large mesodermic cell which, according to Staff, forms the ventral lips of the nephridial funnel; *M.T*, teloblast of mesodermic band—the two dotted lines indicate the hinder part of this band which curves outwards so that it does not lie in the plane of the section; *neph.st*, nephridial string of cells.

HIRUDINEA

The development of Hirudinea is interesting for two reasons, first, because it establishes the Annelidan affinities of this group, which we might be inclined to doubt if we were acquainted only with the adult structure, and secondly, because it was on the embryos of Leeches that Whitman made the first studies of "cell lineage."

We take the development of *Nephelis*, one of the Gnathobdellidae, as type, because the embryology of this form has been worked out in recent times by Sukatschoff (1900, 1903). The egg divides as usual into four macromeres and these bud off a first quartette of micromeres. These micromeres increase by division and form the head blastema. Of the second quartette of micromeres apparently only one member is formed, viz. 2d, the first somatoblast, though the statement that cells are budded inwards from the first quartette of micromeres to surround a stomodaeum or larval oesophagus looks doubtful; possibly renewed investigation will show that these cells are the missing members of the second quartette, and are budded from the macromeres directly. 2d is a large cell quite equal in size to its sister, the residual macromere 2D. From these two cells, four cells of micromeric dimensions are formed at the vegetative pole of the egg.

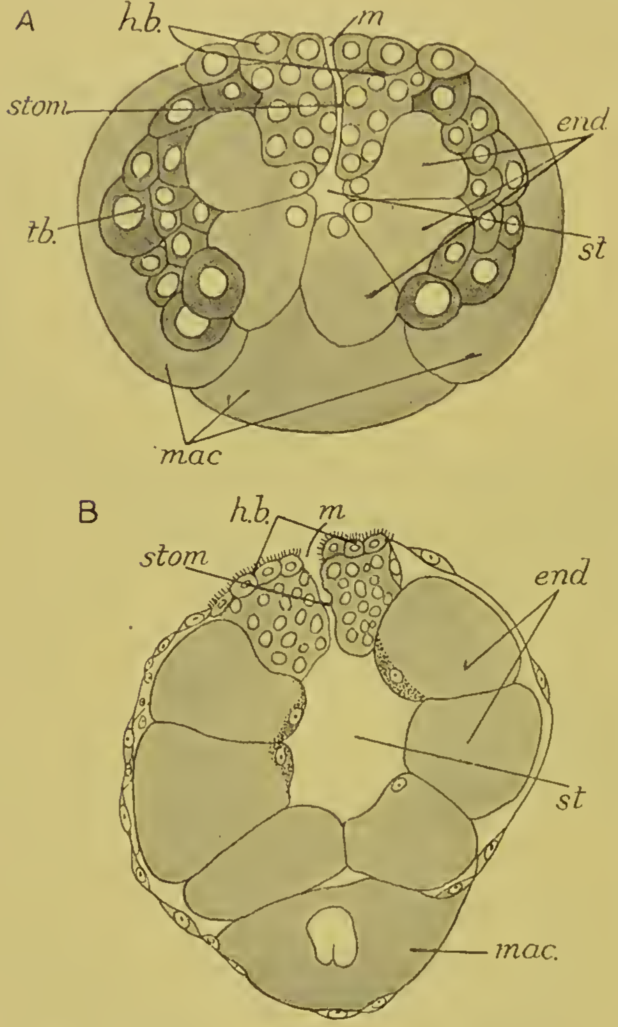


FIG. 119.—Two longitudinal sections of embryos of *Nephelis vulgaris*. (After Sukatschoff.)

A, optical longitudinal (frontal) section of late embryo; B, optical longitudinal (sagittal) section of larva just after escape from the cocoon; *end*, cells (derived chiefly from 3D) forming definitive endoderm; *h.b*, head blastema; *t b*, trunk blastema; *m*, mouth; *mac*, degenerating macromeres; *st*, stomach; *stom*, stomodaeum.

Probably we may interpret this statement thus:—from 2d, $2d^2$ is budded off, which divides into $2d^{21}$ and $2d^{22}$, whilst from 2D, 3d is budded off, sole representative of the third quartette, and this divides into $3d^1$ and $3d^2$. Whether this interpretation is justified or not, we may say at once that these "vegetative micromeres," as we may term them, give rise to the posterior ectoderm and the external circular muscles. They form in fact part of the **trunk blastema.**

The endodermal epithelium does not arise as in other Annelida from the fourth and fifth quartettes of micromeres and from the equal division of the residual macromeres, but by the budding of a single cell from the residual macromere 2B, and of several cells from the residual macromere 4D. The residual macromeres in the quadrants A, B, and C, after having undergone the divisions recorded above, give rise to no more cells; they become smaller and smaller as development goes on, and are finally absorbed. The cells destined to form the lining of the alimentary canal lie between them, and the remains of the macromeres are thus found outside the alimentary canal; a contrast to the condition of affairs obtaining in the Rhyncobdellidae as represented by *Clepsine*, where the endodermal cells are budded from the surfaces of all four macromeres and surround them.

Thus, in one group of leeches the endoderm is formed from practically only one macromere, and lies inside surrounded by the four macromeres, whilst in another group of leeches, the Rhynchobdellidae, including *Clepsine* (*Glossiphonia*) and its allies (Fig. 120), the endoderm is formed from all four macromeres and lies outside them. We conclude that both forms of development are modifications of a primitive type, such as is seen in *Polygordius* and most Polychaeta, in which the residual macromeres are directly converted into the endodermal epithelium; and we are reminded of somewhat similar differences in endoderm formation between Siphonophora and other Hydrozoa, amongst Coelenterata (Chap. IV), and between *Planocera* and *Yungia* amongst Platyhelminths (Chap. V).

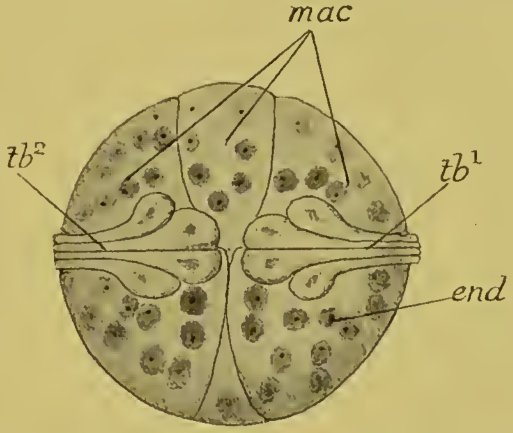


FIG. 120.—A fairly advanced embryo of *Clepsine* (*Glossiphonia*) seen from behind. (After Whitman.)

end, cells forming definitive endoderm on the outer surface of *mac*, degenerating macromeres; *tb*¹, trunk blastema of right side; *tb*², trunk blastema of left side.

In *Nephelis*, after the endoderm is formed, a transverse row of ten cells can be discerned at the hinder end of the embryo. Of these, two are situated more internally than the rest, and these two are the teloblasts of the true coelomic mesoderm and owe their origin to the division of 4d, the sole member of the fourth quartette to be formed. The remaining eight owe their origin to the division of the four "vegetative micromeres" mentioned above. Of the eight, the two nearest the mid-ventral line are termed **neuroblasts,** because they give rise by budding to strings of cells which will form the ventral nerve-cord. Outside these are two cells termed "**nephridioblasts,**" which give rise to strings of cells which separate into groups as the body of the embryo leech lengthens, and form the nephridia. Outside these again are the **myoblasts,** which similarly give rise to cells which form the external circular muscles, whilst finally the two most external cells give rise to strings of cells which broaden out and form the posterior ectoderm. All these strings of cells taken collectively constitute the trunk blastema, it will be thus seen that the development of the trunk blastema is almost identical in character in *Criodrilus* and *Nephelis.*

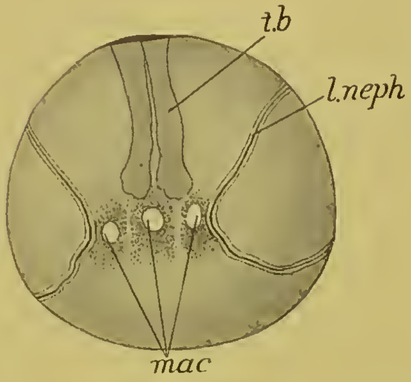


Fig. 121.—Hinder view of a well developed larva of *Nephelis vulgaris.* (After Sukatschoff.)

Letters as before. In addition, *l.neph*, larval nephrida.

A few of the nephridia which are formed at first grow to relatively enormous dimensions, and function as excretory organs during development (Sukatschoff, 1900). These larval nephridia later disappear, and one is involuntarily reminded of the fate which befalls pronephros of Vertebrata (Figs. 121 and 122).

At first the head blastema is widely separated from this trunk blastema by a large expanse of bare macromeres: in a typical annelidan embryo this expanse would be taken up by the prototrochal cells, which are absent here in accordance with the absence of a free larval stage. The strings of cells forming the ectoderm of the trunk blastema rapidly grow forward, beneath them are the two deeper strings formed by the budding of $4a^r$ and $4d^l$, the mother cells of the mesoderm which have arisen by the division of 4d. The trunk

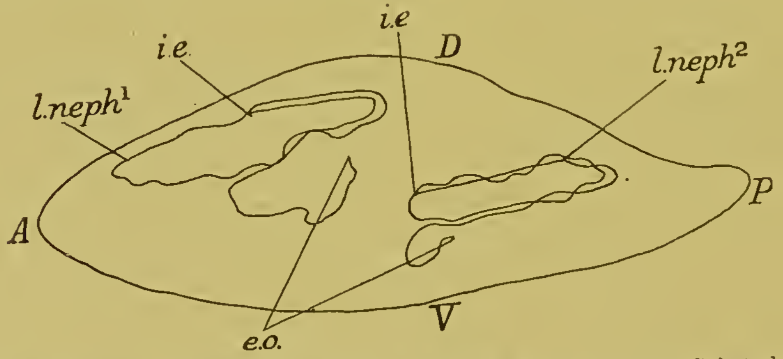


Fig. 122.—Larva of *Nephelis vulgaris* viewed from the side. (After Sukatschoff.)

l.neph^1, the anterior, and *l.neph*^{2}, the posterior larval nephridium; *e.o*, external opening of one side of nephridium; *i.e*, internal blind end of nephridium; *A*, anterior end of larva; *D*, dorsal surface of larva; *P*, posterior end of larva; *V*, Ventral surface of larva.

blastema at first meets the head blastema ventrally, then it subsequently extends up at the sides of the embryo and envelops the dorsal region.

Long before this the mesodermic bands have developed within them a **double series of coelomic cavities.** These rapidly meet one another above the nerve-cord and form the **ventral sinus,** they then extend upwards at the sides of the alimentary canal and meet above it forming the **dorsal sinus.** The septa, which at first would naturally separate each coelomic sac from those anterior and posterior to it on the same side, disappear in the region of the dorsal and ventral sinuses, but laterally they thicken and form the great mass of connective tissue which makes up the "**parenchyma**" of the leech, and the cavities of the coelom between them are thus reduced to narrow slits.

We may note that in *Clepsine* (*Glossiphonia*) the dorsal angles of the coelomic sacs, as they extend upwards towards the mid-dorsal line, bear a row of cells called "**cardioblasts.**" These cells when they meet their partners (viz. when the right one meets the corresponding left one) form the wall of the **dorsal blood-vessel**; the lumen of the vessel arises from the fusion of a string of vacuoles formed in the cells. This dorsal blood-vessel is absent in the Gnathobdellidae.

ALLIED FORMS

In old classifications of Annelida a group termed Gephyrea appears. This group is now totally broken up into three distinct phyla, of the Echiuroidea, Priapuloidea, and Sipunculoidea. Of these the last named possess a Trochophore larva and a most interesting development, which will be dealt with later on, but which is most assuredly not Annelidan in character. The development of the Priapuloidea is entirely unknown.

The Echiuroidea, however, possess a development which proves them to be modified Polychaeta. The embryo starts free-swimming life at a post-trochophoral stage comparable to that in which *Nereis* and its allies begin their larval life. There is a well-marked trochophoral "head," followed by a worm body with numerous closely adpressed somites, which, however, do not develop parapodia. There are well-developed protonephridia, and an apical plate and prototroch, a ventral ciliated groove and larval muscles. The segments disappear in the adult, which exhibits a simple undivided coelom, and a nerve-cord with no ganglionic thickenings.

AFFINITIES OF ANNELIDS

We have now completed our survey of the various types of Annelid development, and it will be seen that they are all easily to be interpreted as abbreviations and simplifications of the long larval history characteristic of *Polygordius.* The primitive nature of

Polygordius, which is deducible from the anatomy of the adult, is fully borne out by a study of its development.

When we turn to the development of the Annelida for light as to the origin of the phylum, the question to be solved resolves itself into the question of how the Trochophore larva is to be interpreted. We have already anticipated the answer to this question in dealing with the Pilidium larva of Nemertinea; we interpreted that larva as representing, in a simplified form, an ancestor which, if it were living now, would be classed as a Ctenophore.

But the Trochophore of *Polygordius*, at the stage when mouth and anus are still not separated, presents an even closer resemblance to a primitive type of Ctenophore than does the Pilidium larva. For the four discrete groups of ciliated cells which have not as yet united to form the prototroch, may be regarded as representing shortened ciliated ribs. Further, Woltereck has shown that beneath the ciliated-apical plate are ganglion cells and fibres, and that from this centre, radial nerve strands extend out to the groups of ciliated cells which are supposed to represent the ribs, just as nerve strands radiate from the polar plate underlying the ribs in Ctenophora.

The likeness of the larval muscles to the contractile strands of Ctenophora has already been dwelt on in the case of the Pilidium, and need not be further insisted on here, but the fact that four, not eight ribs are developed in the Trochophore need not disturb us, especially in view of the fact (see p. 92) that the eight ribs of the Ctenophore are represented in the embryo by four radiating streaks of small cells, and that it is therefore independently probable that in some ancestral Ctenophore there would be four, not eight ribs.

We should thus have three distinct larval types, *viz.* Müller's larva, the Pilidium, and the Trochophore, all representing, with more or less modification, a group of ancestral Ctenophora from which sprang the Polyclada (and through them all Platyhelminthes), the Nemertinea and the Annelida.

The Polyclada are essentially ground Ctenophora which glide over the substratum instead of swimming freely through the water, and Ctenoplana seems to be a modern Ctenophore beginning to undergo a change similar to that undergone by ancestral Ctenophores when they became Polyclada.

As to the nature of the changes which the ancestral Ctenophore underwent in becoming a Nemertinean, we shall not hazard an opinion until the metamorphosis of those worms has been more thoroughly analyzed.

Woltereck, however, attempts to sketch the course of the changes which the Trochophore underwent in becoming an Annelid. He supposes that as it grew older the animal developed the habit of dropping to the bottom and taking to a burrowing habit, whilst still retaining its free-swimming habits during its youth. As a consequence the trochophoral ciliated cells were rubbed off, and the gap in the ectoderm thus caused was closed before and behind by proliferations of cells from the intact ectoderm. In this way he accounts for that characteristic feature of development, the casting off of the prototroch and the union of the head and trunk blastema. The segmentation of the mesoderm and coelom he brings into connection with the wriggling method of progression which replaced ciliary gliding.

It is worthy of note that on this hypothesis, segmentation can only have appeared after the burrowing life was assumed, and the appearance of segments in the free-swimming larva, which is seen even in *Polygordius*, must be regarded as a "telescoping" of development in comparison with what actually occurred in the history of the race. To similar conclusions we are driven by the study of almost all classes of larvae, so there is nothing unlikely in Woltereck's theory, which we may indeed provisionally adopt as by far the best solution of the problem of the origin of the Annelida which has as yet been offered.

We have, however, another problem to face. There appears in the Annelida for the first time, an organ widely distributed in the animal kingdom, over whose nature and origin many battles have been fought. We refer to the **coelom,** often aptly termed by the German authors the **secondary body-cavity** in order to distinguish it from the space which first appears in the embryo termed the segmentation cavity or **blastocoele**; the segmentation cavity is termed by the Germans **the primary body-cavity,** and it forms the space intervening between skin and gut in the Trochophore.

Now the investigations of the American authors show, that the cells forming the wall of the coelom always originate by the division of a single cell 4d, whose sisters 4a, 4b, and 4c, enter into the formation of the endodermic wall of the gut, while 4d itself contributes cells to the gut wall. It is, therefore, reasonable to suppose that 4d itself at one time formed part of the endoderm, and that the adult mesoderm is of endodermic origin. The final demonstration of this belongs to Shearer (1911), who has shown that in *Eupomatus* 4d actually forms part of the endodermic wall of the larva for a whole day after the free-swimming life has begun.

But what changes in successive generations of adults can we suppose to be represented by the separation of a gut-cell from its neighbours, and by its proliferation to form a mass of cells which later become hollowed out to form a cavity? By far the easiest and most natural suggestion is that the process in the larva represents the separation of a pair of endodermal pouches or of a single bilobed pouch from the gut, which eventually became completely shut off from the main gut and devoted to other uses. This interpretation is borne out in the strongest manner by the actual origin of the coelom in other groups where it occurs, and when we are treating of the Echinodermata the question will be discussed more fully.

The alternative theory which was originally put forward by Meyer (1887) is, that the separation of the mesodermic cells from the gut wall represents an outward migration of the primitive genital cells, and that in the ancestral form the genital cells formed compact packets lying at the sides of the gut, and at maturity burrowed their way out as they do in Nemertinea. In later stages of the race it is supposed that after the main mass of cells were dehisced, a peripheral layer was retained and formed the wall of a hollow sac, thus constituting the primitive coelomic sac, which on this supposition was a "**gonocoele.**" It is further supposed that in course of time what were originally genital cells became modified into longitudinal muscles and excretory yellow cells, both of which in Annelida are formed from the coelomic lining.

Now this latter theory seems to fit in well with certain facts. It serves to connect the Annelida and the Nemertinea, and it is undoubtedly true that at the period of sexual maturity in *Polygordius* and in many Polychaeta the coelomic cavities become absolutely blocked up by the mass of genital cells which have been proliferated from their walls. But it is a little disconcerting to find that, whereas, according to theory, the coelomic cavity should not appear till after the main mass of the genital cells has been dehisced, and that the surviving cells should then be converted into peritoneum and peritoneal muscles, in actual development, as a matter of fact the cavity appears first and the peritoneal muscles are differentiated a long time before there is any trace of genital cells. Further, when these genital cells finally do make their appearance and are dehisced, far from the worm taking on a new lease of life, which, according to the theory, must have constantly occurred in some ancestral stock, the animal dies and all its tissues disintegrate. Why the ancestral animal should wait to form muscles till the main purpose of its life has been fulfilled, and what it used for muscles in the meantime, are questions very difficult to answer on this theory.

The theory, however, as we have already said breaks down when other groups of the animal kingdom are studied, unless we are prepared to assume that a fundamental organ with essentially constant character like the coelom, originated from totally different rudiments in different groups, a view which would be subversive of all the recognized principles of reasoning in comparative anatomy. When finally we consider, as all zoologists allow, that the Annelida are derived from Coelenterata, and when we observe that in Scyphozoa, Actinozoa, and Ctenophora, the primitive gut tends to be divided into a central digestive portion and peripheral branches whose walls give rise to muscle cells, and in some of which the genital cells ultimately appear, it seems to us that there can be but one opinion as to which theory of the coelom is the more inherently probable; indeed it is only in consequence of the myopic concentration of attention on the facts of development in a limited number of groups, and the neglect of the facts of development in other groups, that the gonocoele theory ever has obtained any vogue.

The most characteristic feature of Annelida, next to the segmented coelom, is the nervous system, consisting of brain, collar, and ganglionated ventral nerve-cord. The brain is, of course, to be compared to the ganglion cells underlying the apical sense-organ in Ctenophora, which have been experimentally proved to act as a co-ordinating centre for the ciliary activity of the ribs. The ventral nerve-cord originates as two longitudinal thickenings of the ectoderm, situated at the sides of the mid-ventral line.

Now in many larvae in the post-trochophoral stage (cf. *Echiurus*, *Capitella*), the mid-ventral line is occupied by a ciliated groove. This groove extends from the metatroch behind the mouth to the telotroch just in front of the anus, and thus it corresponds roughly to the portion of the blastopore which closes in the process of separation of primitive mouth from primitive anus. This process in the history of the race must have been an extremely gradual one, and while the undivided opening was in the figure-of-eight stage the whole circuit of its lip was probably fringed with cilia, whose activity would assist in the seizing of food. Just, then, as there is a ring-nerve underlying the prototroch, so we might expect to find nerve fibres underlying this ciliated border, and out of these nervous strands we may suppose the ventral nerve-cord to have been built up. If such a circum-oral nerve existed in the original creeping Ctenophore it would certainly be connected with the nerves radiating from the apical centre, and one pair of these connections may have persisted as the nerve collar.

The nephridia—here essentially similar to those of Nemertinea and Platyhelminthes—are a new acquisition. The original function of getting rid of excreta would be naturally concentrated in the ectoderm. When, owing to increase in size and muscular activity, the excretory surface of the ectoderm became insufficient for this purpose, ingrowths and infoldings would take place which would increase its surface and its efficiency, and these ingrowths we suppose to have given rise to the primitive nephridia; and their embryonic history bears out this view.

It would be an extremely interesting thing to investigate the excretory processes in Ctenophora, for thus it is possible that the first fore-shadowings of primitive nephridia might be discovered. But by no means all the excretory work in a Coelenterate is performed by the ectoderm, since the endodermal cells also get rid of some excreta into the gut cavity, whence it is ejected to the exterior. This function would still be carried on by the lateral pockets of the gut, when they were separated from the main axial portion as coelom. Thus the coelomic fluid would tend to become charged with excreta, and so it would have to be periodically got rid of. This would be facilitated by the formation of coelomic pores, such as actually occur in the earthworm, and it seems to have also been accomplished by the fusion of the coelomic wall with the tubes of the ectodermal nephridia, a process which Goodrich believes to have given rise in some cases to the internal funnels of these nephridia, when they occur. In many cases also the genital duct, which originated as a coelomic pore, is utilized for this purpose, and in this way the larger trumpet-shaped nephridia of certain groups, such as the Arenicolidae, may have been formed.

According to the view thus outlined the Annelida would be another instance of a degenerate bottom-living off-shoot from a primitive pelagic group of animals.

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CHAPTER VIII

ARTHROPODA

Classification adopted—

Trilobita.	**Pantopoda.**
Prototracheata (Onychophora).	**Myriapoda.**
Crustacea.	**Insecta.**
Arachnida.	**Tardigrada.**

THE great group of the Arthropoda comprizes at least two-thirds of the species of animals now existing, besides a large proportion of those extinct forms whose remains are preserved as fossils. Of the existing species belonging to the group the greater number are included in the enormous classes of Crustacea, Arachnida, and Insecta. The Myriapoda is a class which can only be described as a lumber-room, since it contains some species which have close affinities with the Insecta and others of widely different relationship. The Prototracheata, Pantopoda, and Tardigrada are small classes including comparatively few species. The Trilobita is a large class, but all the species comprizing it are now extinct and are known only from their fossil remains.

It is generally conceded by zoologists that an Arthropod is merely a further development of the annelidan type of structure. The two groups Arthropoda and Annelida agree in having their bodies built up by a metameric repetition of similar segments, and in the structure of the central nervous system. The most primitive of living Arthropoda are the Prototracheata or Onychophora; they possess a series of so-called nephridia metamerically arranged which correspond to the coelomiducts of Annelida, and they are on this account excluded from the Arthropoda altogether and reckoned as Annelida by some naturalists.

The true relationship which Prototracheata, and through them the rest of the Arthropoda, sustain to the Annelida was made clear by the classic researches of Sedgwick on the development of *Peripatus capensis* (1885–1888). We cannot select this or any other species of *Peripatus* as a type for special description because of the extreme rarity of the animals belonging to this genus, but it is necessary to give a brief account of the principal points elucidated by Professor Sedgwick, in order that we may have a correct apprehension of the significance of the development of other Arthropoda.

PROTOTRACHEATA (ONYCHOPHORA)

The embryo of *Peripatus capensis* passes through its entire development within the oviduct of the mother, and it is born in a form in which it already exhibits all the essential features of the adult. The egg is very minute and, like the eggs of many Insecta, is of an elongated ellipsoidal shape, its longest axis measuring about ·4 mm. It is telolecithal, that is to say that there is a darker area with a minimum of yolk and a maximum of cytoplasm situated at one end of the shortest axis of the ellipsoid, whereas the rest of the egg is paler in colour and richer in yolk. Segmentation is complete; the first four segments formed by the first two cleavages are of the same size, but the third cleavage separates off four smaller darker "animal" cells from four larger paler "vegetative" cells. The first-named by rapid division give rise to the ectoderm, the latter to the endoderm.

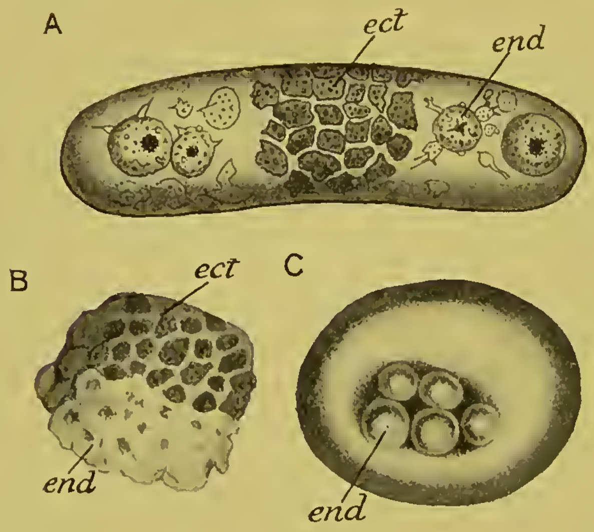


Fig. 123.—Stages in the segmentation and the gastrulation of the egg of *Peripatus capensis*. (After Sedgwick.)

A, conclusion of segmentation. The ectodermic cells form a small cap resting on the endoderm cells which are loosely dispersed within the egg membrane. B, the endodermic cells are contracted so as to form a compact mass, and the ectodermic cells have begun to grow over them (epibole). C, the covering-in of the endoderm cells is almost complete—a few endoderm cells protrude through the blastopore. *ect*, ectodermal blastomeres; *end*, endodermal blastomeres.

When segmentation is completed, the endoderm consists of a number of larger cells loosely connected one with another by strings of cytoplasm which occupy most of the space within the egg membrane, the ectoderm on the other hand forms a cap of small closely aggregated cells which are also connected together by filaments of cytoplasm (Fig. 123 A).

Sedgwick justly attached considerable importance to these connecting filaments, and held that they upset the popular conception of a cell as an isolated unit, and of a Metazoon animal as a mere collocation of such units, or as a colonial Protozoon. He was inclined to regard a multinucleate Protozoon, such as *Actinosphaerium*, as giving a better idea of the common ancestor of Metazoa. Most colonial Protozoa are found, however, when closely examined, to exhibit similar strings of cytoplasm connecting together the various individuals which make up the colony, and so the opposition between the two views tends to disappear.

The mass of scattered endoderm cells undergoes contraction, its units being drawn closely together, so that it forms a compact group of cells, and then the ectoderm grows over its sides and completely invests it, leaving only a small area in the centre of the vegetative pole uncovered. At this spot the large rounded endoderm spheres protrude for a time (Fig. 123 C). Soon a cavity is formed in the centre of the endodermic mass, by the formation of vacuoles which coalesce with one another. This cavity, which is the **archenteron,** opens to the exterior by an aperture, the **blastopore,** in the centre of the uncovered area of endoderm, and so the process of **gastrulation** is completed.

The **gastrula,** which had become nearly spherical, now again elongates, and the blastopore becomes elongated also. Behind it there appears a darker area which seems to be an area of rapid proliferation in the endoderm, this is named by Sedgwick the **primitive streak.** From this area there is produced a crescentic mass of cells lying beneath the endoderm, the two horns of which grow forwards at the sides of the blastopore and constitute the two **mesodermic bands.**

In the meantime the elongated blastopore becomes divided by a constriction into two apertures, the anterior of which persists as the **mouth** whilst the posterior remains as the **anus.** The mesodermic bands then became divided into blocks termed **somites,** in each of which a cavity, the **coelomic cavity,** appears (Fig. 124).

For some time the blastopore is considerably less in length than the embryo, so that there is a prae-oral as well as a post-anal gut, or to put it in another way, there is a short ventral surface and a very long arched dorsal one. The prae-oral and post-anal gut finally disappear owing to the greater relative growth of the ventral surface.

The reader will not fail to observe that up to this stage there is a remarkable general resemblance between the development of *Peripatus* and that of an annelid. The formation of a cap of small ectoderm cells resting on larger endoderm cells and gradually investing the latter by the process termed **epibole**; the division of the blastopore into mouth and anus; the formation of mesodermic bands from endoderm cells in the posterior lip of the blastopore, and their division into metamerically arranged somites, in each of which a cavity appears;—all these are features which have become familiar to us in our study of the development of Annelida.

But from this stage onwards distinctively arthropodan features make their appearance. The rudiments of **appendages** appear as pairs of protrusions of the ventral ectoderm arranged metamerically behind one another in correspondence with the somites; the first to appear are the **antennae** which are at first situated at the sides of the mouth, but which later, along with the corresponding somites, shift forwards to a prae-oral position; the other appendages develop in order from before backwards. Into each rudimentary appendage an outgrowth of the corresponding somite with its coelomic cavity extends. Then the endodermic tube shrinks away from the ectoderm and leaves

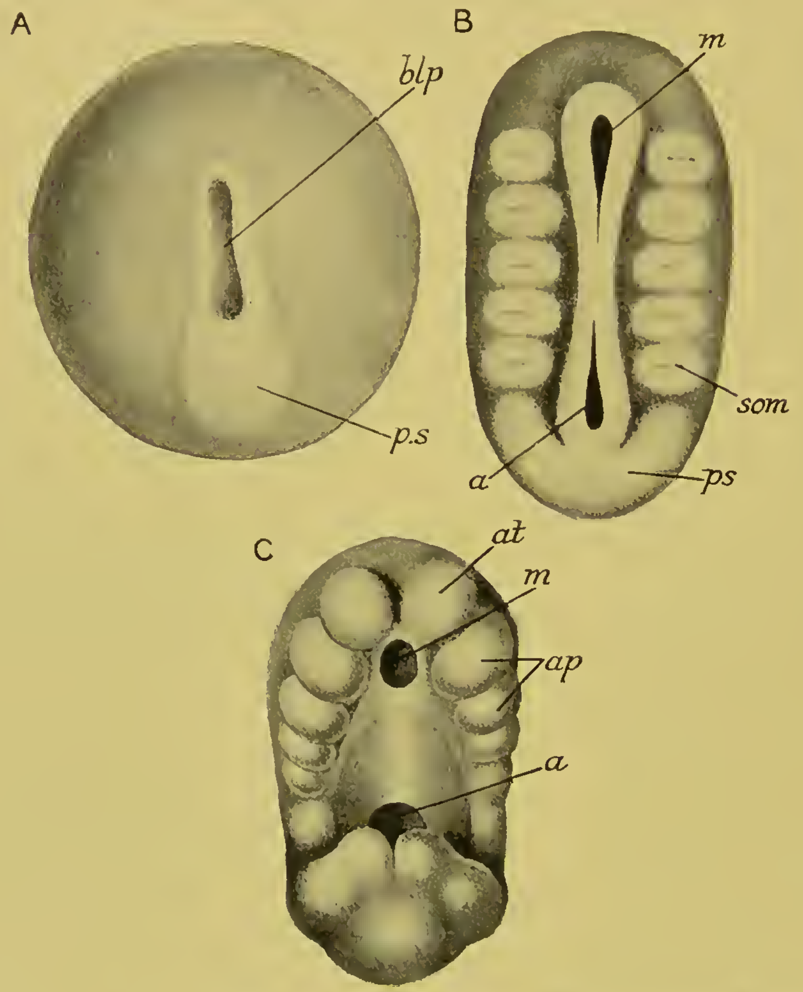


FIG. 124.—Stages in the division of the blastopore and the formation of the mesoderm of *Peripatus capensis*. (After Sedgwick.)

A, the blastopore elongated but unconstricted; the primitive streak is seen behind the blastopore. B, the blastopore has just divided into mouth in front and anus behind. The mesodermic bands have been formed and have already budded off somites in front. C, the embryo has become concave ventrally. The appendages are beginning to grow out from the somites. *a*, anus; *ap*, appendage; *at*, rudiment of antenna; *blp*, blastopore; *m*, mouth; *p.s*, primitive streak; *som*, somites.

spaces which eventually form the body-cavity of the adult, a cavity which is totally distinct from the coelom and is termed the **haemocoele** since it becomes filled with blood (Gr. *haema*, blood). It corresponds exactly to the **blastocoele** or primary body-cavity of the Annelid larva. Of these spaces three primary ones may be distinguished, namely, one median ventral and two dorso-lateral (Fig.

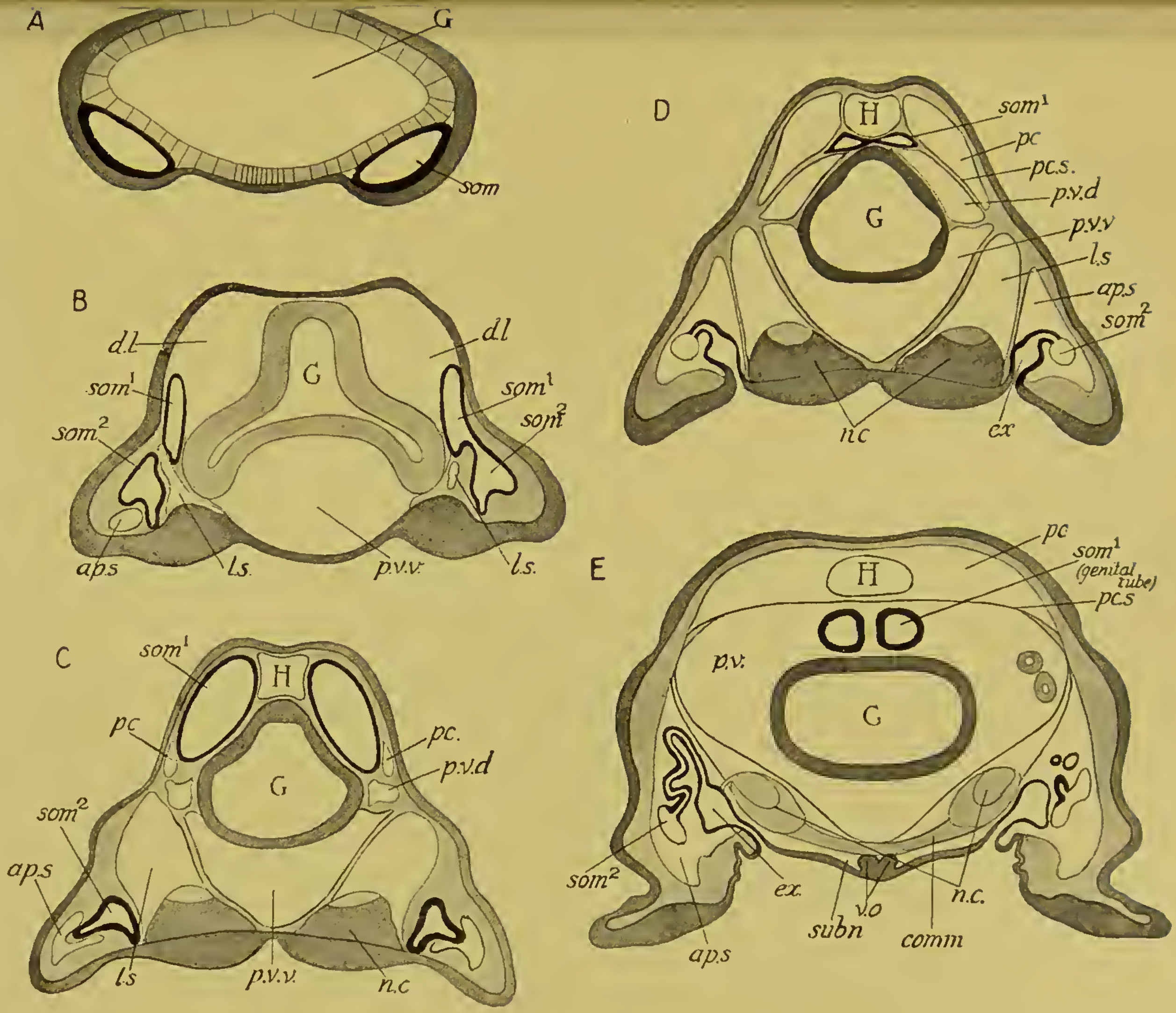


FIG. 125.—Diagrammatic transverse sections through the bodies of embryos of *Peripatus capensis* of various ages in order to illustrate the mutual relationships of haemocoele (primary body-cavity) and coelom (secondary body-cavity). (After Sedgwick.)

A, section through an embryo before haemocoele has appeared. B, section through an embryo in which the somite is just dividing into dorsal and ventral portions. C, section through an embryo in which the somite is completely divided into dorsal and ventral portions, and in which the heart is formed. D, section through an embryo in which the excretory organ is formed, and in which the pericardium is formed, as a result of the flattening out of the dorsal division of the somite. E, section through the genital region of an adult *Peripatus*. *ap.s*, appendicular division of haemocoele; *comm*, transverse commissure connecting a pair of ganglia of the nervous system. *d.l*, dorso-lateral division of haemocoele; *ex*, excretory organ—so-called nephridium; *G*, gut; *H*, heart (formed by coalescence of the two dorso-lateral divisions of the haemocoele); *l.s*, lateral sinus of the haemocoele; *n.c*, nerve-cord; *pc*, pericardium; *pc.s*, pericardial septum; *p.v.d*, dorsal rudiment of general perivisceral cavity; *p.v.v*, ventral rudiment of general perivisceral cavity; *p.v*, general perivisceral cavity formed by the coalescence of dorsal and ventral rudiments; *som*, cavity of the somite, *i.e.* coelom; *som*^{1}, dorsal division of the somite; *som*^{2}, ventral division of the somite; *sub.n*, sub-neural sinus of the haemocoele; *v.o*, ventral organs.

125 A). By the further shrinkage of the endoderm the two dorso-lateral spaces fuse into one median dorsal space which subsequently forms the cavity of the **heart.** Inasmuch as this space is wedged in between the dorsal apices of a pair of somites, it corresponds both in origin and position to the dorsal blood-vessel of Annelida.

Meanwhile each somite has become divided into a dorsal portion lying at the side of the median blood-space, and a ventral portion lying in the base of the corresponding appendage. The walls of both portions, but especially of the latter, give rise by proliferation to a great mass of cells which fills up the appendage and clings to the side of the gut. In this mass other blood-spaces make their appearance. First appears a space in each appendage which embraces the tip of the ventral division of the coelom and forms the cavity of the leg in the adult. This we may term the **appendicular sinus.** Then comes a space nearer the mid ventral line, above the spot where the nerve-cord is formed. The nervous sytem arises in the typical annelidan manner as two ventral band-like thickenings of the ectoderm which remain widely separated in the middle line, but which meet one another in front of the mouth and behind the anus. The brain arises as two thickenings of the prae-oral lobe where these bands meet in front.

The blood-space above each half of the nervous system forms the **lateral sinus** of the body cavity of the adult, and it remains separated from the appendicular sinus and also from the median ventral blood-space by a strand of cells. Above it, at the sides of the gut and external to the dorsal divisions of the coelom, two other spaces appear at each side.

The dorsal division of the coelom, in most of the segments of the body, collapses and forms a flat plate of cells from which the side of the heart and one half of the **pericardial septum** are formed. One pair of the spaces which lay externally to these parts of the coelom, meet above the plates of cells which result from the collapse of the coelomic cavities and form the **pericardium**; the other pair meet above the gut and form the dorsal division of the general body-cavity. This dorsal division of the haemocoele coalesces with the median ventral space and forms the **general perivisceral cavity** of the adult. The ventral division of the cavity of the somite (*som*^{2}, Figs. 125 C and D)—*i.e.* the true coelom—persists as a thin-walled vesicle from which a coiled tube, the **excretory organ,** the so-called "**nephridium,**" grows out and, fusing with the ectoderm on the inner side of the leg, forms there a pore which is the external opening of the nephridium. Finally the nervous system separates from the ectoderm, forming two parallel nerve-cords, and between them and the ectoderm, of which they originally formed a part, a **sub-neural sinus** is formed.

The ectodermic thickenings from which the nerve-cords have broken away, remain for a long time visible, and are termed by Sedgwick **ventral organs.** They gradually approach each other in the mid-ventral line, and it has been surmized that they are a last reminiscence of the **ventral ciliated groove** which extends between mouth and anus in many Annelid larvae.

In certain of the hinder segments of the body the dorsal divisions of the coelom, after giving rise to the lateral walls of the heart and to the pericardial septum, do not utterly collapse, but retain narrow cavities. The somites belonging to several successive metameres fuse with one another so as to form two longitudinal tubes which constitute the **genital organs** (Fig. 125 D). In the penultimate segment the division of the somite into dorsal and ventral portions does not take place; the excretory organ which belongs to this segment forms the lower portion of the **genital duct**, whilst the upper portion of the same duct is formed by the undivided coelom belonging to that segment.

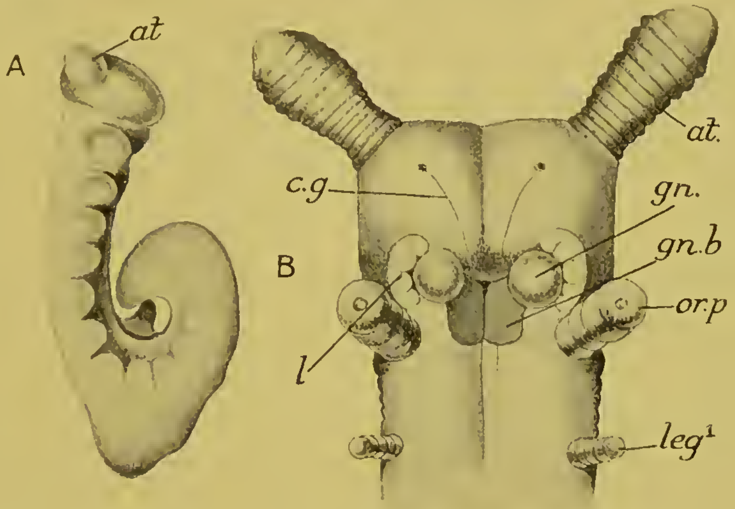


FIG. 126.—The formation of the appendages in the embryo of *Peripatus capensis*. (After Sedgwick.)

A, side view of an embryo in which the appendages are in process of formation, to show the ventral concavity and the dorsal hump. B, ventral view of the head of a much older embryo, to show the cerebral groove and the lip which surrounds the buccal cavity. *at*, antenna; *c.g*, cerebral groove; *gn*, gnathite (jaw); *gn.b*, swelling at base of jaw; *l*, lip enclosing buccal cavity; *or.p*, oral papilla.

It remains to be added that a **stomodaeum** and **proctodaeum** are formed by ingrowths of ectoderm round the lips of the original mouth and anus, which displace these openings inwards; that on the under side of the ectodermal thickenings, which give rise to the brain, two deep pits are formed termed the **cerebral grooves** (*c.g*, Fig. 126 B) which later become closed off from the exterior, and form for a time hollow appendages of the brain; that the outer buccal cavity which envelops the jaws is formed by the growth of a semicircular fold (*l*, Fig. 126 B); that the **salivary glands** are the excretory organs belonging to the jaw segment, from the tubes of which glandular pouches grow out, which project backwards; and finally, that the **slime glands,** which open on the **oral papillae** and secrete the silk with which *Peripatus* spins its web, are of ectodermal origin, as are also the **crural glands** which open on the inner side of the legs external to the openings of the excretory tubes. The **tracheae** develop from simple ectodermal ingrowths which arise very late in development. The **eyes** are simple and formed like the eyes of Mollusca, as vesicles which become closed off from the exterior. A cuticular lens is secreted by the cells of the anterior wall, and the cells forming the posterior wall become the visual cells. The whole organ resembles the "ocellus" of an Insect larva and the cuticular lens may be compared to the "glass body" of the latter (see Fig. 219).

From this necessarily brief and condensed sketch of Sedgwick's results, it will be seen that the change from the annelid to the arthropod type of structure must have been accompanied by a suppression of the coelom and an enlargement of the blood-spaces, the latter forming the functional perivisceral cavity of the adult, while remnants of the former persist in the end-sacs of the excretory organs and the cavities of the generative organs.

This change was also accompanied by an intensification of the secretion of cuticle, and it is just conceivable that this intensification of the secretory powers of the ectoderm entailed the other changes which supervened. If chitin be allied to uric acid, as has been asserted, and if the production and casting off of chitin can be likened to nitrogenous excretion, then we may understand how the coelomic wall, which had previously undertaken a considerable portion of this function, might become relatively unimportant and might tend to dwindle and disappear.

In *Peripatus* the chitinous cuticle is thinner and more flexible than in any other known Arthropod, and in no other Arthropod is a continuous series of "nephridia" retained. In all others the cuticle is thicker and the "nephridia" are reduced to one or a very few pairs; in some cases they seem to be absent altogether. Cuticle and "nephridia" seem therefore to vary in development inversely to one another, and since increase in cuticle seems to entail decrease in "nephridia," it may well be that the same factor has led to the decrease and disappearance of the coelom.

CRUSTACEA

Classification adopted—

(*The new terms invented by Calman* (1909) *have not been universally adopted. They are given in brackets.*)

Order	Group	Subgroup	Calman
I. Phyllopoda	Branchiopoda Cladocera		
II. Cirripedia			
III. Ostracoda			
IV. Copepoda			
V. Malacostraca			
Leptostraca			(Phyllocarida)
Anaspida			(Syncarida)
Stomatopoda			(Hoplocarida)
Arthrostraca	Isopoda Anisopoda Amphipoda		(Peracarida)
Cumacea			(Peracarida)
Schizopoda	Mysidacea		(Peracarida)
Schizopoda	Euphausidacea		(Eucarida)
Decapoda	Macrura	Penaeidea Caridea Nephropsidea Loricata	(Eucarida)
Decapoda	Anomura Brachyura		(Eucarida)

When we now turn to survey what is known of the development of other Arthropoda we find that Insects and Arachnida exhibit, clearly and obviously, a comparatively slight modification of the type of development exemplified by *Peripatus.* But Crustacea have a development which is not so obviously referable to this type. One or two Crustacea are said to have total segmentation of the egg. The best known case of this is the Penaeid shrimp *Lucifer* as described by Brooks (1882). With these exceptions the eggs of all Crustacea, Myriapoda, Insecta, and Arachnida have incomplete segmentation, and all, including those which have total segmentation, have the peculiar disposition of yolk known as **centrolecithal.**

The eggs of *Peripatus capensis* and of allied species are the only Arthropodan eggs which could properly be described as telolecithal. In a centrolecithal egg the yolk is densest in the interior of the egg, and it is surrounded by a skin or rind of cytoplasm. Often the nucleus of the ripe egg is situated near the centre in a sort of island of cytoplasm, but when it divides the daughter nuclei wander outwards and take up their places on the exterior; consequently a segmentation of the egg results which is apparently total but is in reality superficial, for the cleavage planes dividing the blastomeres from one another extend only a limited distance inwards, so that internally all the blastomeres merge in an unsegmented mass of yolk. If this superficial segmentation occurs only on one side of the egg—*i.e.* if the daughter nuclei migrate only to one part of the surface—the segmentation becomes **meroblastic**, although, as we shall see, this meroblastic segmentation differs most markedly from that found in Cephalopoda, which is derived from the teloleeithal method of development.

ASTACUS FLUVIATILIS

We select as type of Crustacean development that of the common river crayfish *Astacus fluviatilis*. A full description of the development of this form is given by Reichenbach (1888), and no such thorough account of the development of any other form has been given before or since.

As every one knows, the eggs are carried throughout their entire development by the mother, attached to her swimmerets by a glutinous secretion. Reichenbach found that when the eggs were removed from the parent they quickly degenerated and died. In the case of the allied genus the Lobster (*Homarus*), it is perfectly feasible to rear the eggs after they have been removed from the swimmerets of the mother, but in order to do this an elaborate arrangement must be provided so as to secure a constant supply of fresh aerated water to bathe the eggs and to ensure that they shall be constantly agitated. Such an apparatus is provided in the various lobster hatcheries built and maintained by the Canadian and United States governments. In default of such apparatus the plan adopted by Reichenbach seems to be efficient and simple, viz. to keep a large number of females carrying eggs in an aquarium, and from time to time to remove a portion of their brood for examination.

By means of very simple arrangements the females can be kept for a long time in a state of perfect health. If the bottom of the tank be covered with only a few inches of water, and provided with an overflow; if a slender stream be kept constantly falling into the tank from a tap; if the tank be provided with a covering of wire-netting in order to prevent the crayfish escaping; and if the whole tank be kept shielded from direct sunlight—then all the conditions will be fulfilled necessary to maintain the crayfish in a healthy condition. They are easily fed on earth-worms, scraps of fish, etc.

All the eggs belonging to any one female are in the same stage of development at one time, but the period required for complete development is a very long one, extending over several months. Thus, by keeping together a large number of females with eggs in very different stages of development, a complete series of stages can be picked out in a very much shorter time than would be required if the eggs of the same female were taken for all stages.

The eggs of the crayfish, like those of most Arthropoda, are very difficult to deal with, as they are composed chiefly of semifluid yolk enclosed in a very tough resistent membrane, and if an attempt be made to remove this membrane, the fluid mass flows out and the egg is destroyed; so Reichenbach recommends the following procedure. The eggs are carefully removed from the parent and are placed in water which is slowly heated to 70° C.; then they are further hardened by being immersed in a 2 per cent solution of bichromate of potash for twenty-four hours; then they are soaked for twenty-four hours in distilled water, which is often changed; and finally, they are transferred to 70 per cent to alcohol. By careful manipulation with needles the thick "chorion" can now be removed and the hardened egg escapes without injury. Reichenbach was accustomed to remove the embryonic rudiment from the egg by means of a sharp knife, then to stain it in picrocarmine, thoroughly dehydrate it and mount it in Canada-balsam; and he also studied the eggs by means of sections cut parallel to and also transverse to the long axis of the embryonic rudiment.

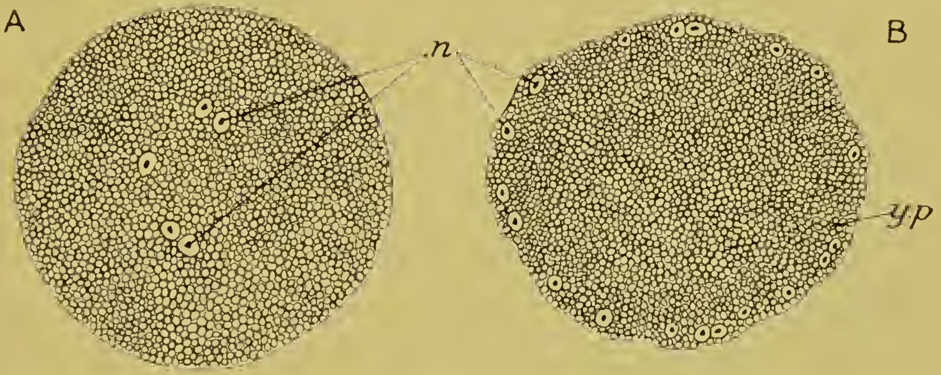


FIG. 127.—Two sections through the developing egg of *Astacus*. (After Morin, from Korschelt and Heider.)

A, stage with few nuclei situated near the centre of the egg. B, stage where the nuclei have reached the surface, and the formation of the primary yolk pyramids has begun. *n*, nucleus; *y.p*, primary yolk pyramid.

As will transpire immediately, there are many points of the greatest interest in the development of the crayfish on which Reichenbach's account throws insufficient light. If, as we hope, this life-history should become the object of renewed investigation, the method of imbedding in celloidin and paraffin, described in Chapter II., would be of the greatest assistance in dealing with eggs like those of the crayfish, which, owing to the number of yolk grains they contain, are exceedingly brittle when hardened.

The earliest stages in the development of *Astacus* were not seen by Reichenbach, whose work begins with the stage when the nuclei which result from the division of the zygote nucleus have reached the surface of the egg, where they form a uniform layer all over its surface. A Russian naturalist, Morin (1886), has, however, figured earlier stages, and from him we learn that the zygote nucleus, as in many other Arthropodan eggs, occupies at first a central position and divides there; and that the daughter nuclei are at first internal but gradually migrate outwards till they reach the surface (Fig. 127).

Reichenbach found that in the first stage observed by him the egg was imperfectly divided by radiating planes into a series of radially arranged pillars, in *each of which was contained one of the daughter nuclei.* These pillars were referred to by previous authors as "**primary yolk pyramids.**" Reichenbach regards them correctly as an imperfect division of the egg into columnar blastomeres; the cleavage planes which separate adjacent pillars correspond to the planes which divide adjacent blastomeres in other eggs. He shows, indeed, that each pillar of yolk is capped on its external surface by cytoplasm containing a nucleus, and is clothed also on its sides with cytoplasm.

In Reichenbach's first stage, then, we have a **blastula** in which the blastocoele is filled with unsegmented yolk. The yolky part of the blastomeres, the yolk pyramids, persist as such for a very short time; the dividing planes disappear, and we are left with a skin of flattened cells surrounding an immense mass of yolk. Such a skin is termed a "**blastoderm.**"

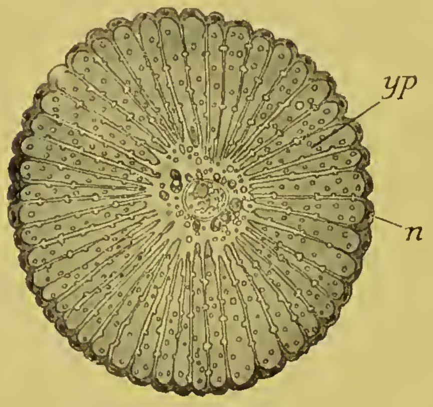


FIG. 128.—Sagittal section through the blastula of *Astacus fluviatilis* to show the primary yolk pyramids. (After Reichenbach.)

Letters as in previous figure.

The formation of the gastrula is initiated by an increase in number of the blastoderm cells on one side of the egg. They press on each other laterally and become columnar in character, and so the "**ventral-plate**" is formed. This ventral plate indicates the future neural side of the embryo. Strictly speaking, all cells within the confines of the plate have not the columnar character; this is confined to five circular areas, in each of which the cells are arranged in elegant concentric curves and in lines radiating from a central point. Of these five areas the two anterior and widest apart are termed the "**cephalic lobes.**" They are the rudiments of the paired eyes and of the cerebral ganglia, and in the centre of each is to be found a pair of cells larger and clearer than the rest.

Behind the cephalic lobes, and situated so close together as almost to touch one another, are two similar areas, which Reichenbach terms the **thoracico-abdominal rudiments**; and behind these again, in the middle line, is a single circular area, the **endodermic rudiment.** At the front border of the endodermic rudiment the cells are engaged in active proliferation, and here they are not in a single layer but in several layers of small rounded cells. This is the point of origin of the **mesoderm.**

In the next stage the areas of the ventral plate which intervene between the five circular areas shrink so as to bring these latter closer together. This shrinkage is almost certainly due to a change in form of the blastodermic cells from a flattened to a more columnar shape. The cephalic lobes, which have increased in size, are brought nearer to each other so that they are only separated by a groove, and they are also approximated to the thoracico-abdominal rudiments. The endodermic disc is indented in its anterior portion by a deep, semicircular groove; this groove is the beginning of the process of **gastrulation** (Figs. 129 and 130), and may be termed the **endodermic groove.**

The mesoderm which lies in front of this consists of a limited number of large cells termed **primary mesoderm,** mingled with a larger number of small cells. The former will give rise to masses corresponding to the somites of *Peripatus*, from which the muscles and probably the genital organs arise; the latter constitute Reichenbach's so-called **secondary mesoderm,** they wander widely and occur everywhere between ectoderm and endoderm, and appear to give rise to blood and connective tissue cells. Reichenbach emphasizes the fact that these cells originate both from ectoderm and from endoderm, but it seems probable that the primary mesoderm has an endodermic origin, while the secondary springs from the ectoderm.

Soon the endodermic groove becomes a complete circle and the periphery of the endodermic disc is invaginated. Just as we have found to be the case in other eggs, the process of invagination can be analysed into (*a*) an increase in the number of cells and (*b*) an inwardly directed cytotaxis. The result of this kind of process is that the centre of the endodermic disc projects for a time as a kind of endodermic button, but as the process continues this button is also carried inwards, and a circular **blastopore** is left where once there was a superficial disc of endoderm. The anterior part of the periphery undergoes the most rapid invagination, and so the endodermic sac projects forward beneath the thoracico-abdominal rudiments.

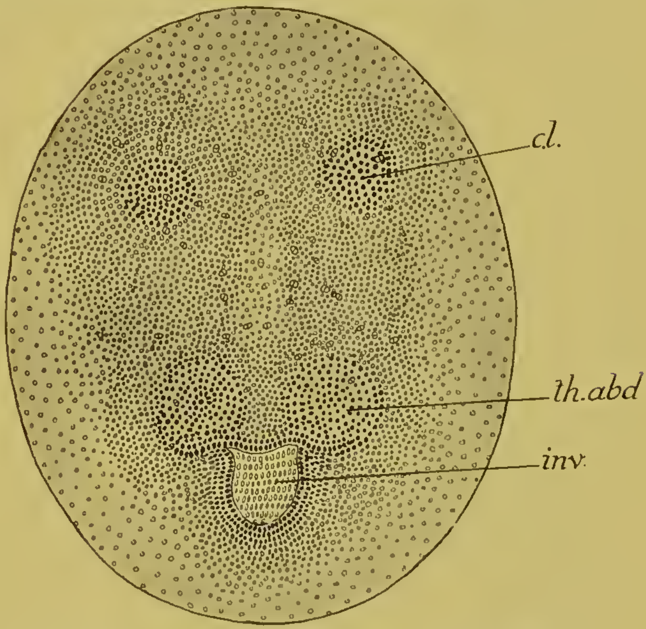


FIG. 129.—Ventral view of an embryo of *Astacus fluviatilis*, the gastrula stage, in order to show the ventral plate. (After Reichenbach.)

c.l, cephalic lobe; *inv*, invaginated area of blastoderm; *th.abd*, thoracico-abdominal thickening.

These rudiments are now connected with one another by a bridge of high columnar cells, and each is also connected with the cephalic lobe of its side by a streak consisting of parallel lines of columnar cells; but between the cephalic lobes there is still a groove of flattened indifferent cells. As a result of these changes we have now a heart-shaped, coherent, ventral plate of columnar cells.

In the next stage the blastopore changes from a circular to an elliptical shape, with its long axis coincident with the long axis of the embryo. The thoracico-abdominal rudiments become thoroughly united with one another in the middle line, and become arched upwards so as to project over the open blastopore and partially conceal it from view. Simultaneously the blastopore begins to close by the lateral union of its sides, the process beginning in front, and its hinder border begins to grow forwards and thus assists in the process of closing.

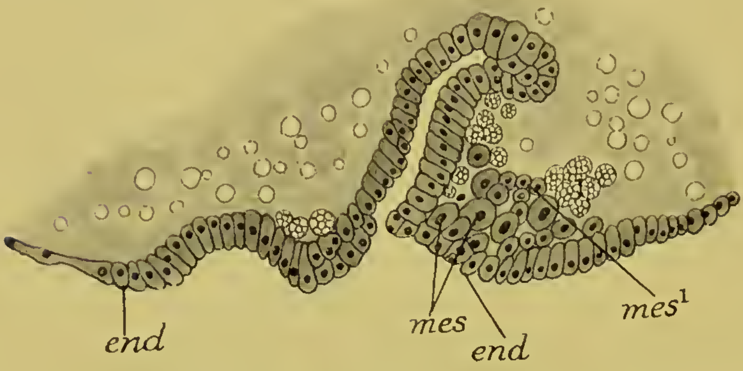


FIG. 130. — Sagittal section through a portion of the embryo of *Astacus fluviatilis* to show the invagination of the endodermic rudiment. (After Reichenbach.)

end, endoderm, the two references to *end* mark the anterior and posterior limits of the endodermic plate; *mes*, "primary" mesoderm; *mes*^{1}, secondary mesoderm.

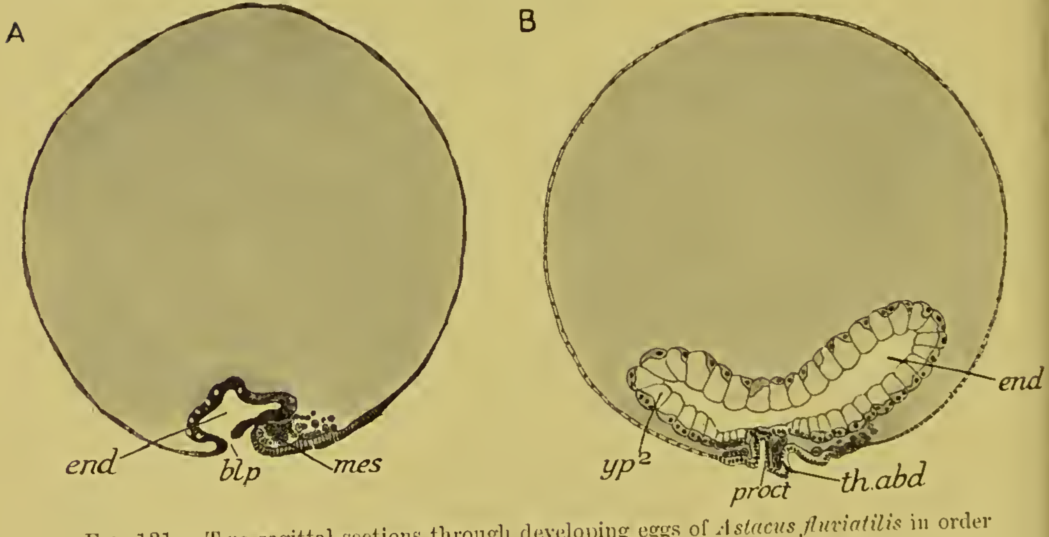


FIG. 131.—Two sagittal sections through developing eggs of *Astacus fluviatilis* in order to show the development of the endoderm. (After Reichenbach.)

A, stage before the closure of the blastopore. B, stage in which the hind-gut has appeared. *blp*, blastopore; *end*, endodermic sac; *mes*, mesoderm; *proct*, proctodaeum opening by anus; *th.abd*, rudiments of thorax and abdomen; *yp*^{2}, endodermic cells swelling up to form secondary yolk pyramids.

Reichenbach's account of this matter and his figures illustrating it are most unsatisfactory. He denies that the backward growth of the abdominal rudiment has anything to do with the closing of the blastopore, and his figures show that this is closed by the union of two flat sheets of endoderm cells, uncovered by ectoderm. Now the lip of the blastopore is a spot where ectoderm passes into endoderm; it is difficult to imagine that in the process of closing there is a dissolution of this continuity, and the suspicion is aroused that if these stages were worked over by the celloidin-paraffin method different results would be obtained. In all probability the dissolution of continuity is due to the method of section cutting.

At this same time the cells which formed the endodermic button and which now form the floor of the endodermic sac become more columnar in shape. This increase in size is due to the fact that they begin actively to ingest the yolk granules; and they continue to do so in successive stages till all the yolk granules, which made up the unsegmented mass in the centre of the egg, are contained in the yolk cells. The endodermic cells increase enormously in length during this process and were termed by the earlier authors the **secondary yolk pyramids**; their growth is, however, little advanced in the stage which we are now discussing.

As the thoracico-abdominal rudiment advances over the blastopore it becomes obviously bilobed, and in the notch between the lobes is seen the last rudiment of the blastopore. In front of this, according to Reichenbach, *i.e.* in the bridge which connects the two halves of the rudiment, a new invagination makes its appearance; it is the rudiment of the adult intestine or **proctodaeum**, which opens by the **anus.** It is by no means improbable that further investigation would show that the proctodaeum arises just where the last vestige of the blastopore disappeared.

At the same time the two cephalic lobes have become connected in their hinder region by a curved bridge of columnar cells. This is the rudiment of the **labrum** or upper lip; behind it, in a slightly later stage, an invagination appears which will mark the position of the **mouth** and of the **oesophagus (stomodaeum)**, but of these there is, at this period, no trace. In the streaks of cells connecting the cephalic lobes and thoracico-abdominal rudiments, three outwardly directed, semicircular thickenings are observable, of which the hindermost pair are the furthest advanced. These are the rudiments of the first three pairs of **appendages**, viz. the **antennules**, **antennæ**, and **mandibles** of the adult.

The mesoderm when last considered consisted of a small number of large and of a large number of small cells. In this stage the large cells form a mass beneath the thoracico-abdominal rudiment, whilst the smaller have extended and spread all over the surface of the ventral plate and form special aggregations in the cephalic lobes and in the lip rudiment.

As the rudiments of the appendages become more marked the ventral plate continues to shrink in size and takes on an oval outline. On the median side of each appendage is to be seen a mass of cells with large clear nuclei; these are the rudiments of the **ganglia** of the **nervous system.** The first of these pairs of ganglia is connected with a similar mass of cells which forms a kind of focal line, surrounded by the concentric parabolic curves of cells which make up the cephalic lobes. This focal mass of cells is the rudiment of the **primary cerebral ganglion** or **protocerebrum,** to which later the **antennulary ganglion** or **deuterocerebrum** adds itself. The two cerebral ganglia are connected by a bridge in front of the labrum. To the compound mass on each side there is added, at a later period, the **antennary ganglion** or **tritocerebrum** (*tr.c*, Fig. 137). The outer part of the cephalic lobe gives rise to the **eye-stalk,** the ectoderm covering which gives rise to the visual cells of the compound eye; at its base there is a deep groove, the cells lining which, in later stages, bud off the cells which form the **optic ganglion.** This groove may be termed the **cerebral groove.**

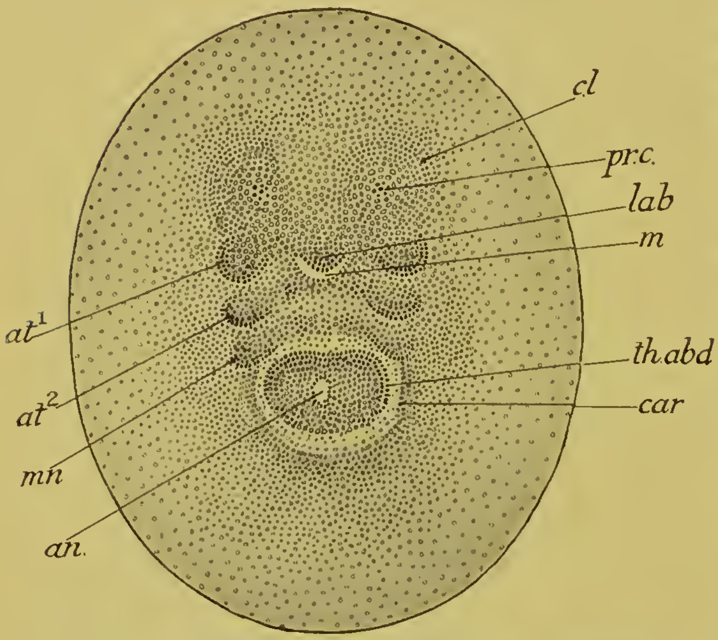


FIG. 132.—The "Nauplius" stage in the development of *Astacus fluviatilis* viewed from the ventral side. (After Reichenbach.)

an, anus; *at*¹, rudiment of first antenna; *at*², rudiment of second antenna; *car*, ridge marking the first trace of the carapace; *c.l*, cephalic lobe; *lab*, labrum; *m*, mouth; *mn*, rudiment of mandible; *pr.c*, protocerebrum; *th.abd*, rudiment of thorax and abdomen.

The **mouth** has now made its appearance as a groove behind the labrum and leads into a narrow **stomodaeum,** which descends vertically towards the endodermic sac but does not yet reach it. Behind the mouth there is found a median groove of ectoderm extending backwards between the ganglia of opposite sides. The cells forming this groove proliferate and form between each pair of appendages a thickening, two or three cells deep, which later enters into the formation of the **transverse commissures** between the ganglia of the double ventral nerve cord (Fig. 133).

The primary mesoderm forms a compact mass, in which, however, some indications of a division into segmental masses corresponding to the appendages are to be seen. This is one of the points on which a renewed investigation is very desirable, because Reichenbach's statements on this point have been overlooked by subsequent workers, and it has been generally assumed that Crustacea are distinguished from other Arthropoda by the non-segmentation of the mesoderm (Balfour, 1880). The **anus** is still situated on the dorsal aspect of the thoracico-abdominal rudiment. It is, however, shifted somewhat forwards as compared with its former position, and will eventually pass into the terminal notch and so on to the ventral surface of the abdominal rudiment; but this does not happen until a later stage has been reached.

Finally, on the surface of the egg, outside the thoracico-abdominal rudiment, there is to be seen a semicircular ridge—very faintly marked. This is the first trace of the **head-shield or carapace.** When this stage of development is reached the ectoderm secretes a thin cuticle which is detached from the surface of the egg before further growth occurs, and we may interpret this as the first moult or **ecdysis,** and as marking the completion of a stage of development.

Now when we survey what is known of the life-histories of other Crustacea we find that, in the majority of Copepoda, Cirripedia, and Ostracoda, and in the more primitive Phyllopoda as well as in a few Schizopoda and Decapoda, the embryo, when it has attained this stage of development, bursts the egg-shell and escapes as a free-swimming larva, to which the name **Nauplius** has been given, and which is distinguished by possessing a large upper lip and only three pairs of appendages. We can scarcely doubt that the formation and exuviation of this cuticle in the embryo of *Astacus* is a reminiscence of a condition when the embryo, at this stage of development, became a free-swimming Nauplius larva—and we regard this as one of the many proofs that the embryonic phase of development is secondarily derived from the larval, and not *vice versa.* We therefore term this stage of development the Nauplius stage.

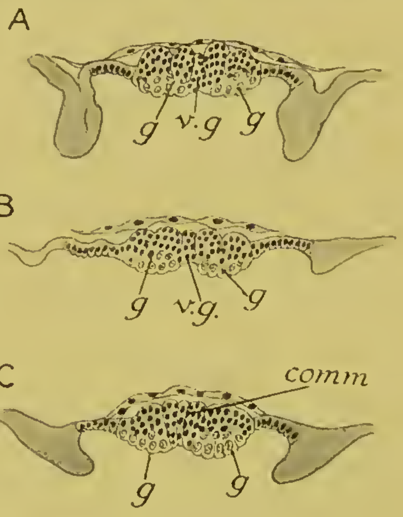


FIG. 133.—Three transverse sections through the developing nerve cord of *Astacus fluviatilis.* (After Reichenbach.)

comm, mass of cells derived from ventral groove destined to form the transverse commissure; *g*, thickening to form one of the ventral ganglia; *v.g*, ventral groove.

Following the Nauplius stage a different form of development begins; the ventral shield, which had been undergoing contraction, begins to grow vigorously, and in the angle where this shield passes into the ventral surface of the thoracico-abdominal rudiment there is found a zone of rapidly growing cells, and as a result of their activity the point of origin of this rudiment is carried farther back. Thus it becomes bent under the ventral shield, just as a crab carries its abdomen permanently bent underneath it.

Then if we turn our attention to the appendages, we find that the original three have become longer; and that the second, which is the rudiment of the antenna, has become bifurcated at the end, which is an indication of the branching of the limb into **exopodite** and **endopodite.** Behind the mandible is the region of the **ventral shield,** which owes its origin to this budding zone; on it are to be found the rudiments of five new pairs of appendages, viz. those corresponding to the **first** and **second maxilla** and to the three pairs of **maxillipedes.** All except the first of these are very faintly marked indeed.

The thoracico-abdominal rudiment has grown in length and has become marked out into segments by grooves. On the dorsal side, just at its point of origin, there is seen an ectodermic thickening. Below this there is a plate-like mass of mesoderm, the ends of which in the next stage become bent upwards and attached to the ectoderm, so as to enclose a space which is the cavity of the **heart** (H, Fig. 135). In the sides of this mesodermal mass, just as in the case of *Peripatus,* irregular cavities appear (*pc,* Fig. 135). These are the rudiments of the **pericardial cavity** and they

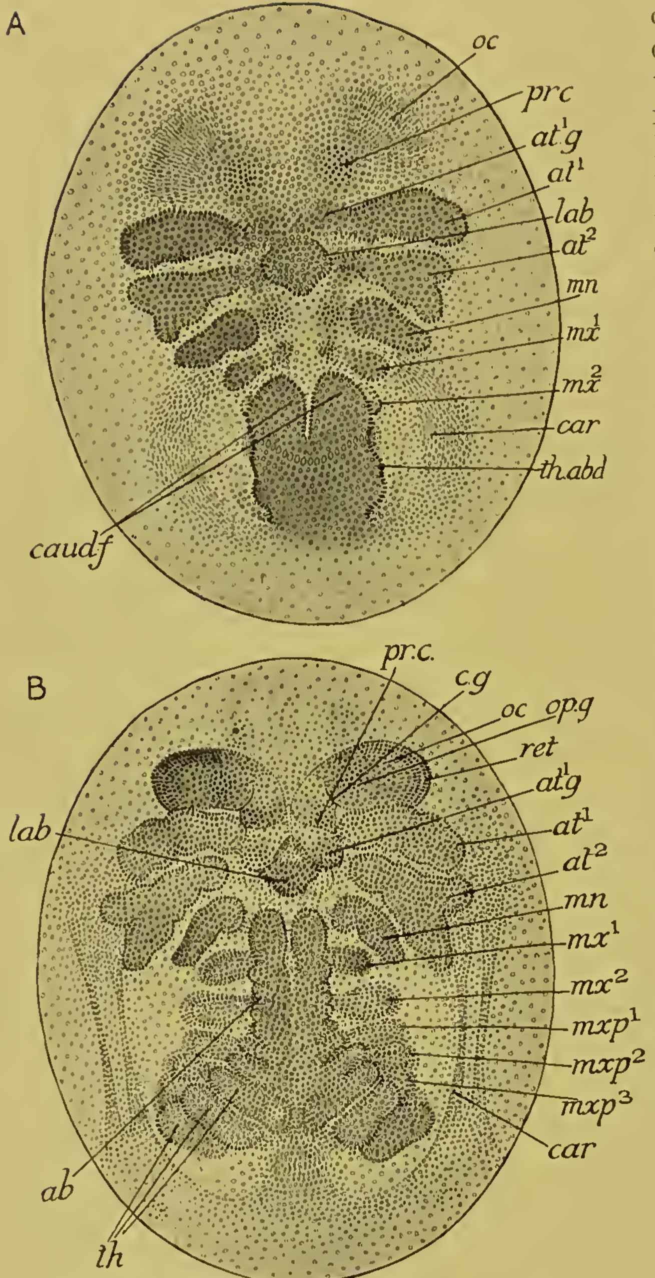


FIG. 134.—Two views of developing eggs of *Astacus fluviatilis* seen from the ventral surface. (After Reichenbach.)

A, stage in which the rudiments of maxillae have appeared, and in which the caudal fork is visible. B, stage in which the rudiments of thoracic appendages are appearing, and in which the abdomen is segmented. *ab*, abdomen; *at*^{1}, first antenna; *at*^{1}*.g*, antennulary ganglion (deuterocerebrum); *at*^{2}, second antenna; *car*, fold which becomes edge of the carapace; *caud.f*, caudal fork; *c.g*, cerebral groove which gives rise to the optic ganglion: *lab*, labrum; *mn*, rudiment of mandible; *mx*^{1}, first maxilla; *mx*^{2}, second maxilla; *mxp*^{1}, *mxp*^{2}, *mxp*^{3}, first, second, and third maxillipedes; *oc*, eye-stalk; *op.g*, optic-ganglion; *pr.c*, protocerebrum; *ret*, retinulae of the compound eye; *th*, rudiments of thoracic appendages.

eventually meet above the heart and separate it from the ectoderm. The primary mesoderm becomes divided into a double series of spherical masses, one pair corresponding to each of the segments into which the thoracico-abdominal rudiment is divided. In these **somites** cavities appear which represent the coelomic cavities of *Peripatus.* At the termination of the thoracico-abdominal rudiment there is a deep indentation and the anus is no longer visible; in other words, the tail is divided into two lobes which are termed the tail lobes or **caudal fork.** The anus has in fact been pushed forwards till it lies in the notch between these lobes, and it then passes on to the ventral surface by the partial fusion of the two tail lobes above it. Just in front of these lobes there is a crescentic area of rapidly growing cells. This is a second budding zone, and it is to its activity that the increased length of the thoracico-abdominal rudiment is due.

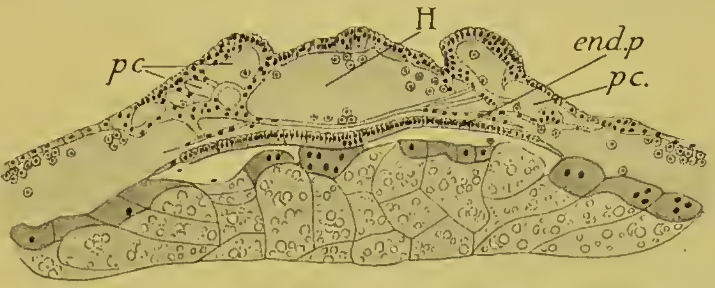


FIG. 135.—Transverse section through the region of the heart in an embryo of *Astacus fluviatilis* in about the same stage as that represented in Fig. 134 B. (After Reichenbach.)

end.p, endodermal plate; *H,* heart; *pc,* spaces destined to form the pericardium.

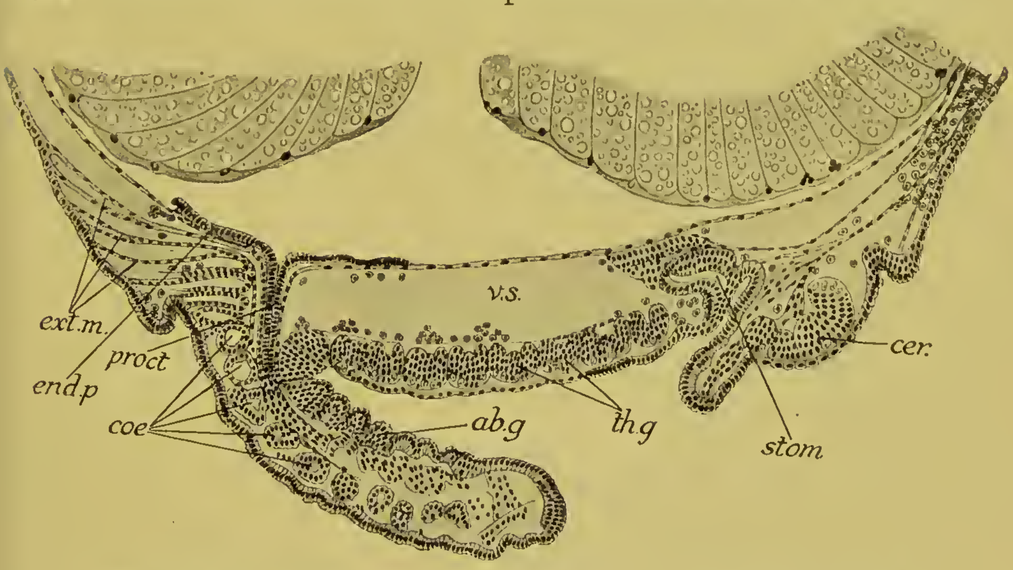


FIG. 136.—Longitudinal section through advanced embryo of *Astacus fluviatilis* parallel to the sagittal direction but to one side of the middle line. (After Reichenbach.)

ab.g, abdominal ganglion; *cer,* cerebral ganglion; *coe,* coelomic cavities; *end.p,* endodermal plate; *ext.m,* extensor muscles; *proct,* hind-gut; *stom,* fore-gut; *th.g,* thoracic ganglia; *v.s,* ventral sinus.

In the next stage the yolk has been completely ingested by the endoderm cells, which have become in consequence very tall and columnar in shape, and the fold which gives origin to the carapace is strongly marked. Beneath it there is a deep groove on each side which gives rise to the **branchial cavity** of the adult. Five additional pairs of appendages, the rudiments of the so-called **ambulatory legs**, the possession of which causes *Astacus* to be reckoned as a Decapod, are developed, and the first and second maxilla and the three maxillipedes have become bifurcated.

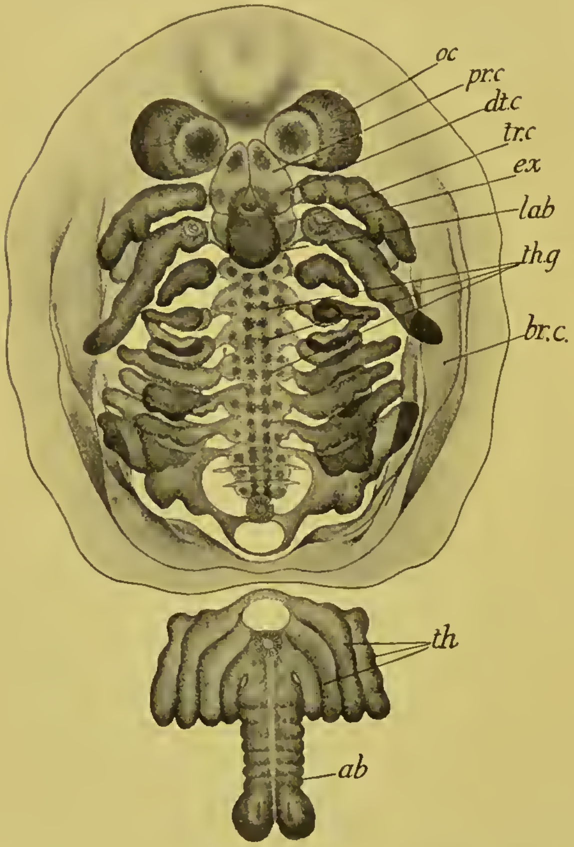


Fig. 137.—Advanced embryo of *Astacus fluviatilis* viewed from the ventral side. The abdomen and hinder part of the thorax are cut off and spread out separately. (After Reichenbach.)

ab, abdomen; *br.c*, rudiment of branchial cavity; *dt.c*, deuterocerebrum; *ex*, opening of excretory organ; *lab*, labrum; *pr.c*, protocerebrum; *oc*, eye-stalk; *th*, thoracic legs; *th.g*, thoracic ganglia; *tr.c*, tritocerebrum.

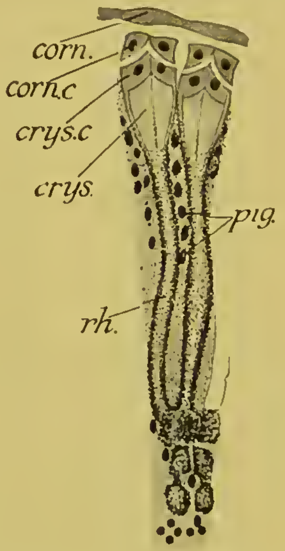


Fig. 138.—Two ommatidia from the eye of a newly-hatched crayfish in longitudinal section. (After Reichenbach.)

corn, cornea; *corn.c*, corneal cells; *crys*, crystalline cone; *crys.c*, crystalline cone cells; *rh*, rhabdome shimmering through the retinula; *pig*, pigment cells.

In the basal part of the rudiment of the antenna a sac appears, which is the rudiment of the **excretory organ** (*ex*, Fig. 137). This sac appears to be similar to one of the coelomic sacs of the abdomen, to it is added a large ectodermic pocket which forms the thin-walled **ureter**.

Soon afterwards the rudiments of the **abdominal feet**, or **pleopods**, make their appearance; and in the bifurcated antennule there is an ectodermic pit to be seen, which is the beginning of the **auditory organ**.

The cephalic lobes now project freely from the surface of the egg as the **eye-stalks**, and the ectoderm cells covering them have become several layers deep. These ectoderm cells then become arranged in radially directed strings, each of which forms an **ommatidium** or eye element; the outermost cells giving rise by their secretion to the **corneal lens**, those beneath them to the **crystalline cone**, whilst from the innermost cells the **retinula** is derived (see Figs. 220–222).

The coelomic cavities of the mesoderm disappear as the primary mesoderm cells form themselves into flexor and extensor muscles (*ext.m*, Fig. 136). The arched dorsal region of the egg begins to flatten in consequence of the gradual digestion and diminution of the masses of yolk stored up in the endoderm cells. The outer ends of these cells, in which the nuclei are situated, gradually separate from the yolky portions. These latter break up into rounded masses and are gradually digested. The first place where the cytoplasm separates from the yolk is in the dorsal surface of the **mid-gut**, just where the proctodaeum impinges on it. Here a flat stretch of epithelium, the "**endodermal plate**," becomes separated from the yolk (*end.p*, Fig. 135). Soon the **rostral spine** begins to be differentiated between the antennules in the head region. The **gills** appear under the branchiostegite as outgrowths from the basal joints of the limbs. The two halves of the caudal fork fuse to form a simple rounded **telson**. The ectoderm everywhere sends inwards solid pegs; they form the supports for the tendons and ligaments of connective tissue which are formed by the wandering cells.

The embryo is now ready to break open the egg-shell and enter upon its free life. For some time the store of yolk in the endoderm suffices; but gradually the extreme convexity of the dorsal hump disappears, as the remaining store of yolk is used up, and the endoderm cells shrink in size. The flaccid endodermic sac becomes indented by folds, and is gradually fashioned into the complex structure of tubes known as the adult **liver**. Its median portion persists as the adult **mid-gut**.

Just before the embryo hatches Reichenbach was able to detect the rudiments of the **genital organs**. These appear in the 14th, 15th, and 16th segments in the dorsal region, and appear to consist of rounded masses of cells, in each of which a lumen appears. The masses seem to be arranged metamerically in accordance with the segments, and at the hinder end of the rudiment of each side there is to be seen a tube, which is presumably the rudiment of the **genital duct**. Reichenbach's imperfect observations, so far as they go, fit in admirably with Sedgwick's results on *Peripatus*.

OTHER CRUSTACEA

We shall now take a brief survey of what is known of the development of other Crustacea, and shall direct our attention to two points: (1) the mode of formation of layers, *i.e.* the differentiation of ectoderm, endoderm, and mesoderm, and (2) the larval history.

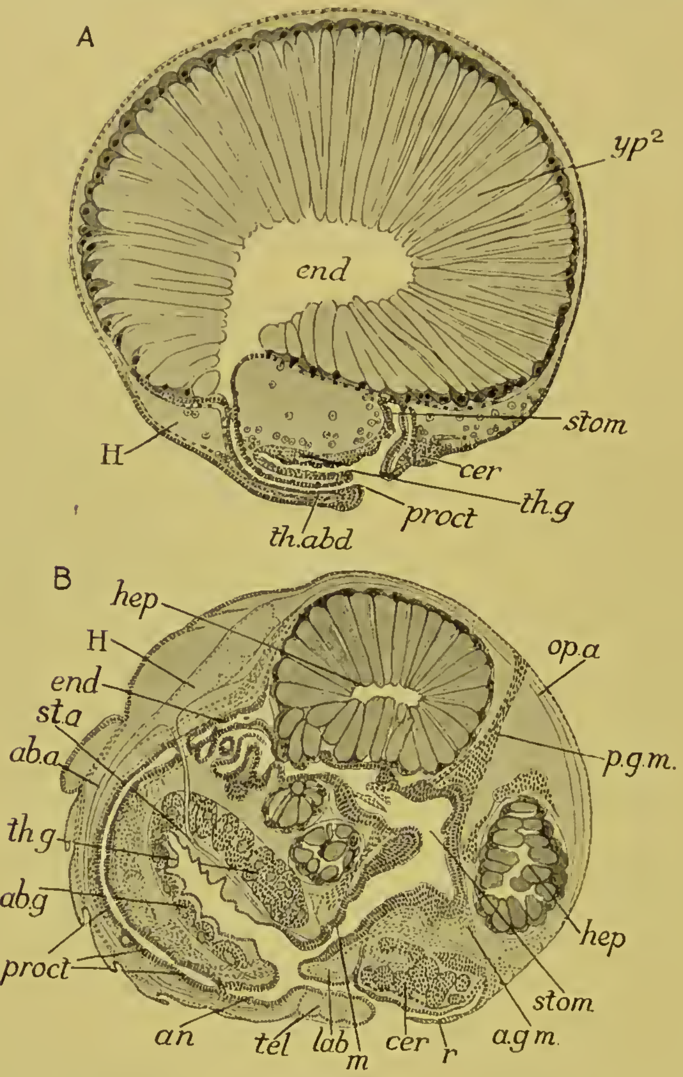


FIG. 139.—Two sagittal sections through advanced embryos of *Astacus fluviatilis*. (After Reichenbach.)

A, stage in which secondary yolk pyramids are complete. B, stage in which the endodermic sac is divided into lobes—the rudiments of the liver tubes. *ab.a*, abdominal artery; *ab.g*, abdominal ganglia; *a.g.m*, anterior gastric muscle; *an*, anus (the reference line goes to a point some little distance inside the proctodaeum); *cer*, cerebral ganglion; *end*, endodermic sac (mid-gut); *H*, heart; *hep*, liver saccule; *lab*, labrum; *m*, mouth; *p.g.m*, posterior gastric muscle; *proct*, proctodaeum (hind-gut); *r*, rostrum; *st.a*, sternal artery; *stom*, stomodaeum (fore-gut); *tel*, telson; *th.abd*, thoracico-abdominal rudiment; *th.g*, thoracic ganglia; *op.a*, opthalmic artery; yp^2, secondary yolk-pyramid.

FORMATION OF LAYERS

With regard to the first point, all the fragmentary knowledge which we possess of the early history of other Crustacean eggs seems to show that they agree in all essentials with the egg of *Astacus* as to the mode in which the layers are differentiated. If we look at the nearer allies of *Astacus,* we find that in *Homarus* (the lobster) the egg is much larger than that of *Astacus,* owing to the presence of a larger amount of yolk, and that the endodermic area is relatively small. The invaginated cells form at first a nearly solid mass projecting into the yolk; they multiply and spread through the yolk, ingesting it as they proceed, and a cavity appears in the interior of the mass filled with disorganized cells. Eventually they reach its surface, and here form an investing layer; thus secondary yolk pyramids are not formed.

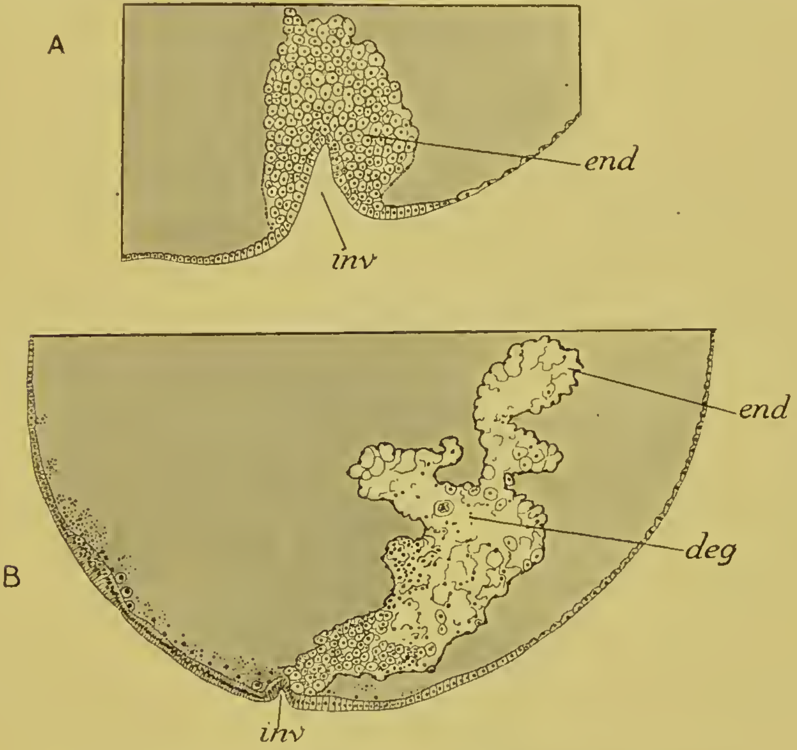


FIG. 140.—Portions of two sagittal sections through developing eggs of *Homarus americanus.* (After Herrick.)

A, stage in which the endoderm cells form a solid mass. B, stage in which the endoderm cells are spreading through the yolk. *deg,* degenerate remains of more central cells; *end,* endoderm; *inv,* cavity of invagination.

Much the same process occurs in the prawn *Palaemon*; but here the endoderm cells become detached from one another and wander through the yolk and eventually arrange themselves in an epithelial layer outside it; when they have reached this position they become columnar but never attain the length of the endoderm cells in *Astacus.* This kind of development seems to be general throughout the Decapoda.

In *Lucifer,* however, as we have seen above, Brooks asserts that the segmentation of the egg is total, that a hollow blastula consisting of relatively few cells is formed, and that an invagination takes place by which an archenteron is formed which is large and occupies most of what was the interior of the blastula.

The development of the Schizopod *Euphausia*, as far as the gastrula stage, has recently been worked out by Taube (1909). Here, as in *Lucifer*, the egg undergoes total segmentation and the blastomeres are all of nearly the same size. In this way a hollow blastula is formed. After the 32-cell stage, however, the cells do not all divide; two remain undivided, and form the rudiment of the endoderm, and these, at the 112-cell stage, pass into the interior of the blastula. The blastopore is surrounded by a ring of special cells, and of these two are said to give rise to the mesoderm.

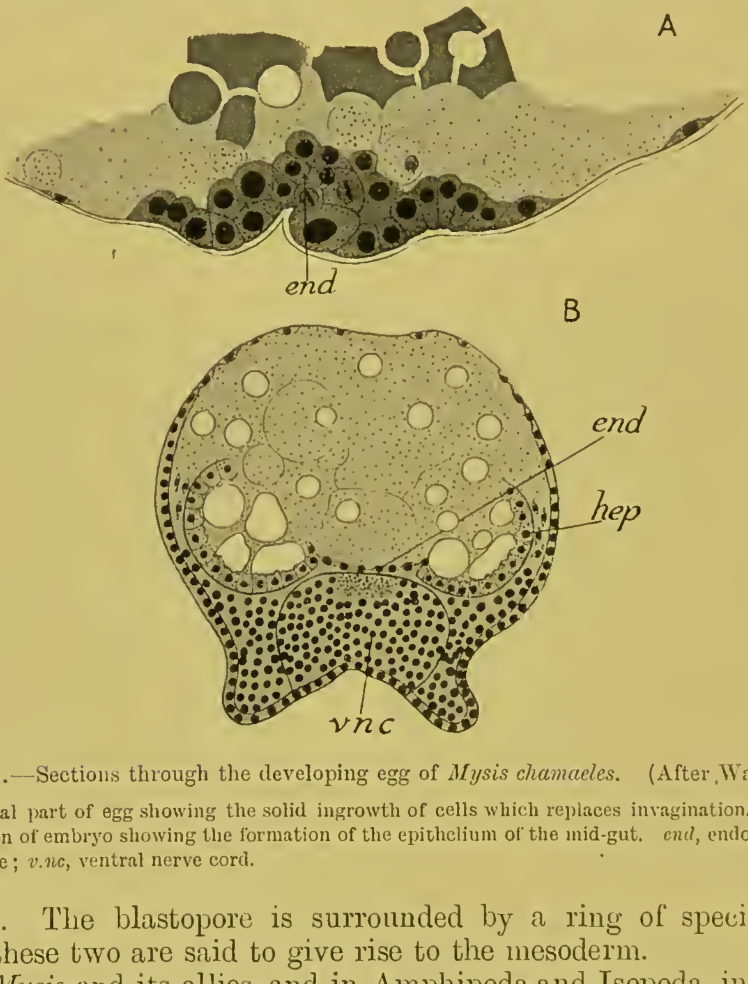


FIG. 141.—Sections through the developing egg of *Mysis chamaeles*. (After Wasbaum.)

A, ventral part of egg showing the solid ingrowth of cells which replaces invagination. B, transverse section of embryo showing the formation of the epithelium of the mid-gut. *end*, endoderm; *hep*, liver saccule; *v.nc*, ventral nerve cord.

In *Mysis* and its allies, and in Amphipoda and Isopoda, in a word in all the Peracarida, however, the invagination is replaced by a solid ingrowth of endoderm cells, and when these detach themselves and wander through the yolk, they form an endodermal epithelium, at first only on the ventral side of the yolk (Fig. 141). Only very gradually does this epithelium extend so as to enclose the yolk on the upper side also. In these cases too we have **meroblastic segmentation**, *i.e.* the zygote nucleus, whether it is in the interior of the egg, as in the Isopoda, or on the surface as in *Mysis*, gives rise to daughter nuclei, which form a blastoderm, at first only on the ventral surface of the egg; only at a later period do cells come to the surface of the yolk on the dorsal side also.

When we descend to the lower groups of Crustacea we find that amongst Phyllopoda the development of the Cladoceran genus *Polyphemus* has recently been worked out by Kühn (1912). In fundamental characters it agrees with that of *Euphausia*; the egg undergoes total segmentation. A 2-cell stage is followed by a 4-cell stage and this by an 8-cell stage in which there are two tiers of four cells, and in which a segmentation cavity or blastocoele makes its appearance. The four cells nearer the animal pole of the egg are larger and clearer than those nearer the vegetative pole, but the latter contain most of the yolk, and in one of them are embedded the remains of the sister cells of the egg, *i.e.* oocytes, which do not ripen, but serve as nourishment. In the 16-cell stage we get two tiers of eight cells each, since every cell except one divides by a meridional cleavage. This exceptional cell is the one of the four situated in the vegetative half of the egg, which has received the remains of the nutritive cells. It divides, not meridionally, but into an upper and a lower cell; the lower contains the remains of the nutritive cells, it is the rudiment of the genital organs, and is termed the **generative cell**; the upper is the **endoderm** cell, and gives rise to the lining of the mid-gut. At the next period of cleavage these two cells do not divide, but all the other cells divide each into an upper and a lower daughter cell (Fig. 142, A). In this way we get in the animal half of the egg two tiers of eight cells, and in the lower half of the egg an upper tier of six cells and a lower tier of six cells. This lower tier lies at the vegetative pole and forms a horse-shoe-shaped group surrounding the endoderm cell and the generative cell. There are thus thirty cells in the egg. Shortly after the endoderm cell divides into right and left halves, thus raising the number of cells to 31.

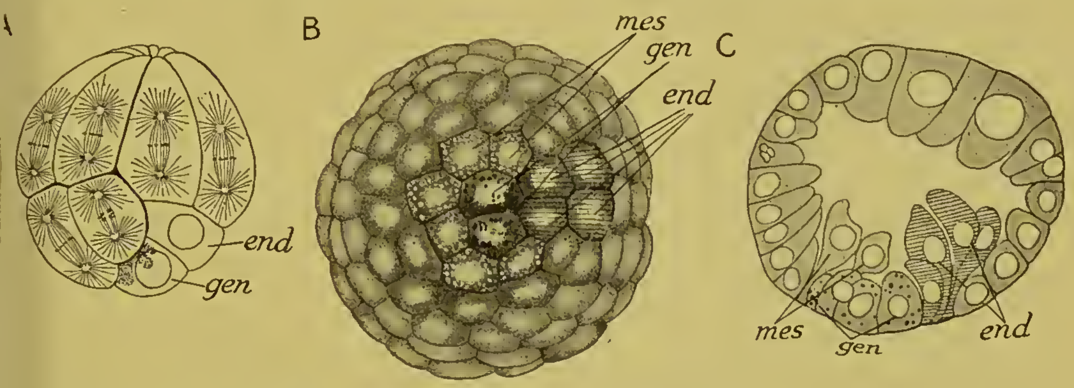


Fig. 142.—Stages in the development of the egg of *Polyphemus pediculus*. (After Kühn.) A, passage from 16-cell stage to 30-cell stage, from the side. B, 118-cell stage, from below. C, sagittal section through a stage of between 236 and 452 cells. *end*, endodermal cells; *gen*, cells of genital rudiment; *mes*, mesoderm cells.

At the next period of cleavage the endoderm cells divide into upper and lower daughters, and the generative cell divides into right and left halves, and in this way a 62-cell stage is attained. The two generative and four endodermic cells do not, however, divide in the following period of cleavage, so that instead of 124 cells we have only 108 in the next stage. In this stage the group of endodermic and generative cells is surrounded by a horseshoe-shaped group of six cells, descendants of the similar group in the 30-cell stage. This group constitutes the **mesoderm** (Fig. 142, B). When the endodermal group becomes invaginated, as a result of inwardly directed cytotaxis, the mesoderm cells are also invaginated (Fig. 142, C). Kühn derives the mesoderm from the ectoderm because in the 30-cell and 62-cell stages the mother cells of the mesoderm are cells which give rise also to daughters which eventually form part of the ectoderm. But this comparison is misleading and unjust. The wall of the blastula is differentiated into regions, an endodermic below, a mesodermic above this, and above this again an ectodermic. The mere fact, that when the blastular wall consists of few cells, mesodermic and ectodermic regions happen to find themselves contiguous to one another so as to be within the territory of one of the few nuclei, is of no importance. The mesoderm of *Polyphemus* corresponds in position and origin to that of *Astacus*. The early differentiation of the genital rudiment is a common feature in animals of small size and short life-cycle.

About the development of the other division of Phyllopoda (*i.e.* Branchiopoda) very little is known. The egg of *Branchipus* is stated to undergo total segmentation, but the inner ends of the blastomeres are said to coalesce into a yolky mass, on the surface of which is a blastoderm. Doubt has recently been cast on this statement, and there is no doubt that it requires reinvestigation.

The development of Copepoda and Cirripedia seems to be fundamentally of a similar type. It may be regarded as a modification of the type described for *Astacus*, a modification which is produced by the diminution in absolute size of the egg, due to the smaller adult size of the species, coupled with the fact that the nucleus and its daughter nuclei are not diminished in the same proportion as is the whole egg. Therefore the amount of nuclear matter relative to the size of the egg is greater in these forms than in Decapoda, and the nuclei are also far fewer in numbers. The result of this is to produce a form of segmentation which might be variously described either as holoblastic or meroblastic, according as one regarded the nucleus which remains nearer the centre of the egg, as either—(1) the nucleus of a huge blastomere whose cell territory includes all the egg which is not marked out into blastoderm, or (2) as a nucleus in unsegmented yolk which has not as yet had its cell protoplasm delimited.

The development of *Lepas* and its allies has been studied by Groom (1894), and the development of *Lepas* in its earlier stages has been studied in great detail by Bigelow (1902). In this case the mother nucleus divides three times successively, and at each division gives off a daughter which migrates to the surface and segregates round itself a blastoderm cell, the pre-existing blastoderm cells also dividing each time. In this way an investment of the yolk by blastoderm cells is effected. At the fourth cleavage the mother nucleus gives rise to a primary **mesoderm** cell in front and then comes to the surface itself as the first endoderm cell. Subsequently both mesoderm and endoderm cells divide into right and left halves, and the endoderm cells withdraw from the surface. This inwardly directed cytotaxis is the process of gastrulation. The cells bordering the blastopore at the anterior end bud off cells which sink inwards; these may be termed **mesectoderm,** and they are perhaps equivalent to the secondary mesoderm of *Astacus.* A **mesodermal band** is formed which extends forwards and upwards, and along its course three outwardly directed transverse grooves delimit the three pairs of appendages of the Nauplius. The dorsal extension of these appendages is in reality the same phenomenon as their outward direction in *Astacus,* and the apparent difference in direction is due to the smaller quantity of yolk in the egg of *Lepas.*

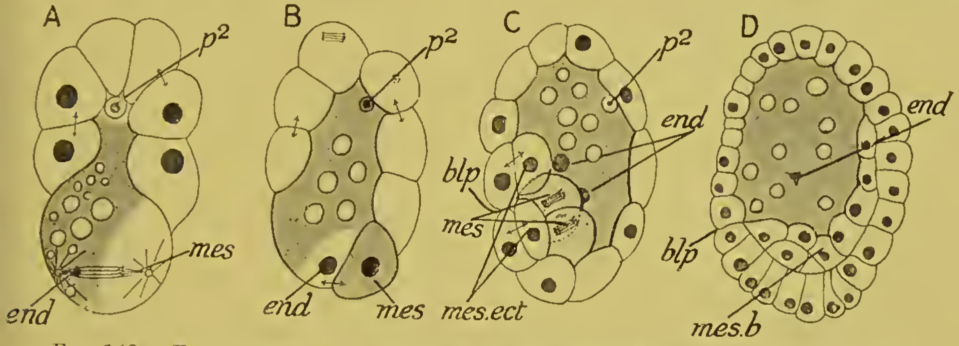


FIG. 143.—Four sagittal sections through the developing eggs of *Lepas anatifera* in different stages of development. (After Bigelow.)

A, 15-cell stage, formation of mesoderm. B, 16-cell stage, mesoderm formed. C, 62-cell stage, formation of mesectoderm. D, 250-cell stage, formation of mesodermic band. *blp*, blastopore; *end*, endodermal nucleus; *mes*, mesoderm; *mes.ect*, mesectoderm; *mes.b*, mesodermic band; p^2, second polar body.

The development of Copepoda, to judge from the somewhat conflicting accounts which we possess, seems to be essentially similar to that of *Lepas.*

A renewed study by modern methods of the development of a primitive form like *Branchipus* would throw a flood of light on the development of Crustacea generally, and perhaps enable us to understand the conflicting accounts given of the development of Copepoda.

LARVAL HISTORY—THE NAUPLIUS

With regard to the larval history, we may take as type the development of the common fresh-water Copepod *Cyclops,* of which various species are found in fresh water all over the world. If females of *Cyclops* carrying egg-sacs are isolated and kept in small shallow glass vessels in pond-water with a little pond-weed, they will live for a considerable time, and the eggs will hatch out into larvae, and these larvae can be reared through their complete development till they attain the adult condition.

When the larvae escape from the egg membrane they have an oval outline, and are provided with a large, swollen, almost square upper lip and three pairs of appendages. Of these, the first pair are inserted in front of the lip, and each consists of a single branch divided into three joints, of which the centre one is the largest. All three carry long hairs at their ends. The second pair of appendages on each side consists of a broad basal piece (protopodite); it carries a long, inwardly directed hook, which nearly meets its fellow beneath the lip. This pair of appendages is postoral. The distal portion of the limb consists of two branches, an exopodite or outer branch, composed of a basal piece and four joints, and an endopodite or inner branch, composed of basal piece and two joints. The basal pieces of exopodite and endopodite are more or less adherent. The third pair of appendages are quite similar to the second, but smaller in size; the protopodite is longer in proportion than in the second pair of appendages, and it has on its inner side a triangular outgrowth, which carries one or two long, inwardly-directed bristles. This, like the corresponding process on the second appendage, is masticatory in function. The exopodite and endopodite consist as before of four and two joints respectively, but the distal joint of the endopodite projects inwards. These three pairs of appendages are moved by long, backwardly-directed muscles, which converge towards and are inserted in a small area in the dorsal integument.

The mouth leads into a vertical oesophagus which is provided with constrictor and dilator muscles. This, from its cuticular lining, is obviously an ectodermal stomodaeum. It opens into the true endodermal gut, which runs backwards nearly to the posterior end of the animal; here it opens by an orifice, guarded by a sphincter muscle, into a short proctodaeum lined by cuticle and derived from the ectoderm. In front of this opening the midgut gives rise to two ventro-lateral pouches, which have an excretory function and are filled with granules of uric acid.

In the base of the second pair of appendages is the opening of a sac which projects backwards at the side of the mouth. This sac, the **antennary sac**, is also excretory in nature, and is homologous with the similarly situated sac in *Astacus*.

The nervous system consists of a praeoral brain, on which rest two simple eyes, and a sub-oesophageal ganglion connected with it by a pair of cords. The nerves for the second and third pair of appendages are connected with the sub-oesophageal ganglion.

The little larva was baptized **Nauplius** by Claus (1858). The name had been previously employed by the Danish naturalist, O. F. Müller, for a later stage in the development, when four pairs of appendages were formed; he imagined this to be an independent organism. When the Nauplius is just hatched it remains still for a few seconds, until its cuticle hardens and becomes strong enough to resist the pull of the muscles. Then it starts on its active career, swimming by a series of darts through the water, each dart being caused by a synchronous backward blow of all the appendages. At each blow of the legs the masticatory hooks seize any food particles that they may encounter and drive them into the mouth; feeding and swimming are thus performed by the same movements.

If we compare this larva with the stage in the development of *Astacus* when a cuticle is first formed, we can see that there is a fundamental resemblance between the embryo in one case and the larva in the other; the difference between the two being, first, that the appendages of the embryo, since they are not functional, are represented by mere stumps; and secondly, that the embryo possesses cephalic lobes, and the rudiments of compound eyes, which are absent in the larva. But the adult Copepod also has no rudiments of compound eyes, and the origin and significance of the compound eye is still an unsettled question.

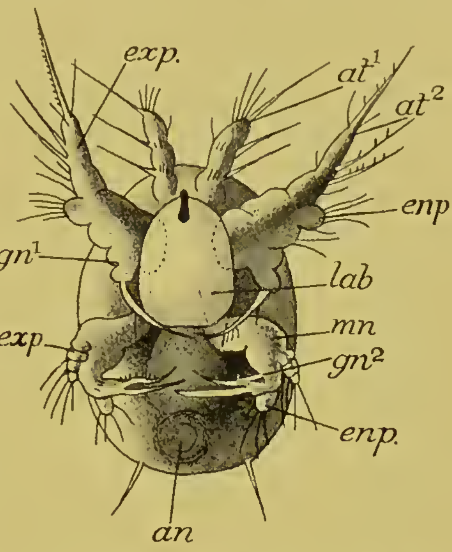


FIG. 144.—The Nauplius larva of *Cyclops canthocarnoides* from the ventral surface. (After Claus.)

an, anus; at^1, first antenna; at^2, second antenna; *enp*, endopodite; *exp*, exopodite; gn^1, gnathobase of second antenna; gn^2, gnathobase of mandible; *lab*, labrum; *mn*, mandible.

The little creature, as it begins to feed, grows, and, like all Arthropoda, can only grow by casting its cuticle. Just like the embryo of *Astacus*, there are two growing regions, one at the posterior end of the animal, which gives rise to a pointed prolongation of the body equivalent to the thoracico-abdominal rudiment of *Astacus*, and one immediately behind the third appendage.

At the end of the first moult the larva passes into what has been called the **Metanauplius** condition, when the small rudiments of the two pairs of maxillae and the so-called maxillipede, or third maxilla, appear behind the large third appendage. They are concealed by it, and hence Claus, in his original communication (1858), came to the astounding conclusion that not only the adult mandibles but the two first pairs of maxillae were derived from the division of the third appendage of the Nauplius. The posterior end of the larva becomes bilobed, the anus is in the bay thus formed, and just beneath the spot where the excretory sacs of the gut are situated there are developed a pair of stumps, which eventually form the first thoracic feet.

By three successive moults the length of the posterior part of the body is increased, and at each moult a new pair of stump-like

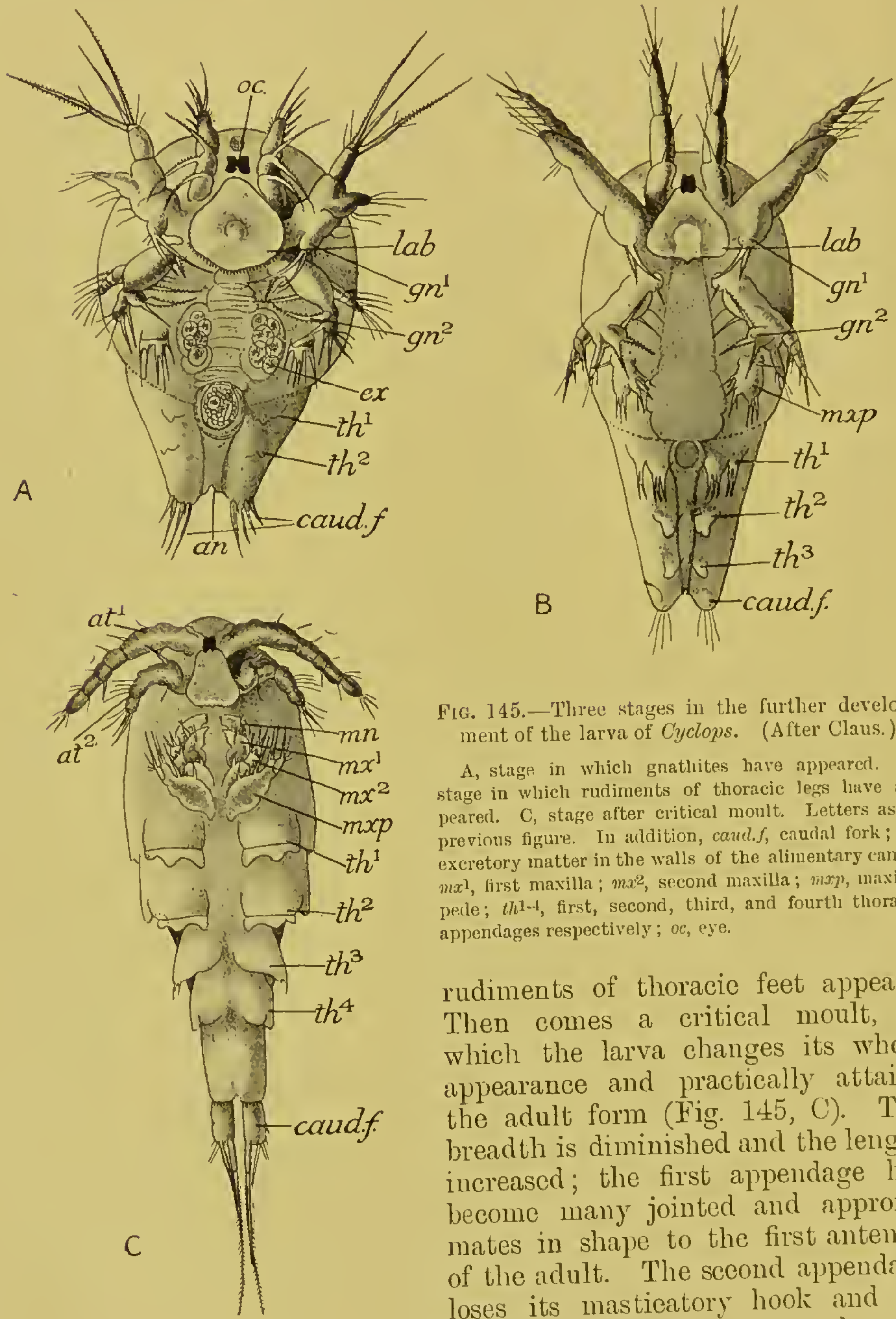
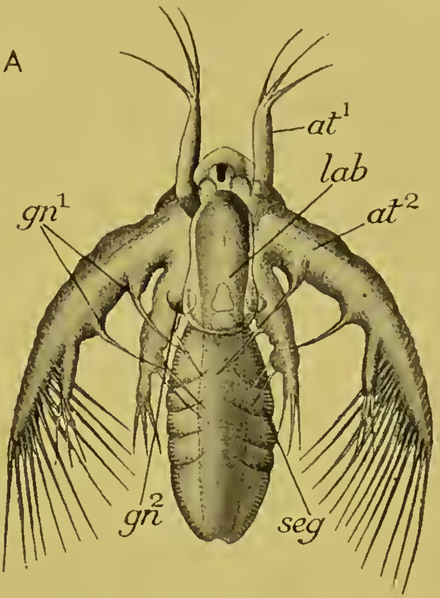


Fig. 145.—Three stages in the further development of the larva of *Cyclops*. (After Claus.)

A, stage in which gnathites have appeared. B, stage in which rudiments of thoracic legs have appeared. C, stage after critical moult. Letters as in previous figure. In addition, *caud.f*, caudal fork; *ex*, excretory matter in the walls of the alimentary canal; *mx*^{1}, first maxilla; *mx*^{2}, second maxilla; *mxp*, maxillipede; *th*$^{1-4}$, first, second, third, and fourth thoracic appendages respectively; *oc*, eye.

rudiments of thoracic feet appears. Then comes a critical moult, at which the larva changes its whole appearance and practically attains the adult form (Fig. 145, C). The breadth is diminished and the length increased; the first appendage has become many jointed and approximates in shape to the first antenna of the adult. The second appendage loses its masticatory hook and its endopodite, and has now become shorter than the first: it becomes the second antenna of the adult. The third appendage loses everything but the basal joint, and so is converted into the mandible. The first two pairs of thoracic legs are each now distinctly divided into two branches, but both exopodite and endopodite are as yet undivided. The bifurcation of the caudal end has now deepened, so that the anus is guarded on each side by a rod-like appendage—one half of the **caudal fork**. In the base of the second maxilla may be seen the sac-like rudiment of the **shell-gland**—the adult excretory organ, whilst the antennary sac has disappeared. The thoracico-abdominal rudiment is now distinctly divided into segments.

At successive moults additional joints are added to the first antenna, the branches of the thoracic legs become jointed, and the posterior thoracic legs



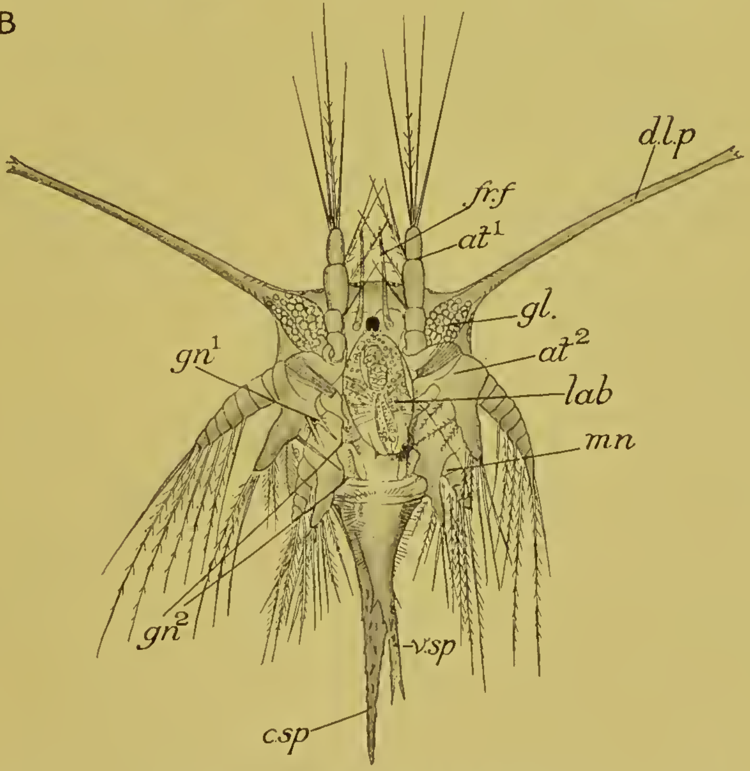


FIG. 146.—Two types of Nauplius larva.

A, the Nauplius larva of *Branchipus stagnalis* (after Claus) combined from two figures. B, the Nauplius larva of *Lepas* (after Groom). Letters as in preceding figure. In addition, *c.sp*, caudal spine; *d.l.p*, dorso-lateral spine; *fr.f*, frontal filament; *gl*, glandular mass in the base of the dorso-lateral spine; gn^1, gnathobase of antenna; gn^2, gnathobase of mandible; *seg*, segments in the thoracico-abdominal rudiment of the Nauplius of *Branchipus*; *v.sp*, the pre-anal spine.

become first forked, then jointed, and so the adult form is attained.

Claus, in the paper cited, has given an interesting sketch of the differences between Nauplii of different species, and one of the most interesting facts which he brings out is that in the larvae of Cyclopsinidae, in which more yolk is contained in the egg, the masticatory hooks on the second and third appendages are absent, and the animal at first takes no food.

When we examine the life history of the Phyllopoda, we find that in the Branchiopoda and in one or two cases amongst the Cladocera, the young animal enters on its free-swimming existence as a Nauplius larva. In most Cladocera, however, the whole development is completed within the egg-shell, and the animal hatches out with all the adult features already developed. The Nauplius larva, when it appears, shows the same general features as the larva of *Cyclops*, but the upper lip is very long and projects backwards, covering the ventral surface.

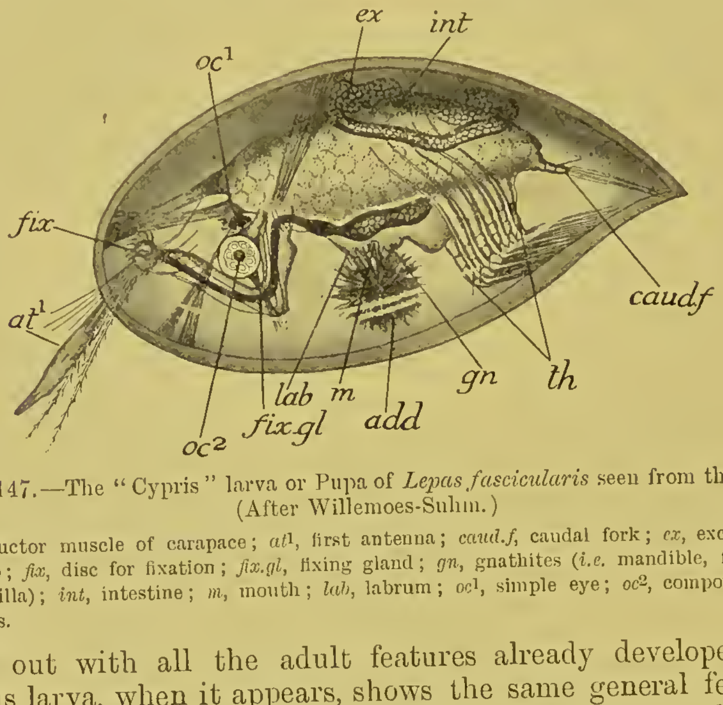


FIG. 147.—The "Cypris" larva or Pupa of *Lepas fascicularis* seen from the side. (After Willemoes-Suhm.)

add, adductor muscle of carapace; *at*^{1}, first antenna; *caud.f*, caudal fork; *ex*, excretory organ (shell-gland); *fix*, disc for fixation; *fix.gl*, fixing gland; *gn*, gnathites (*i.e.* mandible, first maxilla, second maxilla); *int*, intestine; *m*, mouth; *lab*, labrum; *oc*^{1}, simple eye; *oc*^{2}, compound eye; *th*, thoracic legs.

If we turn to the Branchiopoda we find that the Nauplius larva is characterized by the great development of the post-oral portion of the body, and by the fact that the third appendages are not forked (Fig. 146, A). After the first moult, before any more appendages appear, the post-oral region becomes marked by a series of four or five transverse grooves, an indication of as many segments, and in the larva of *Apus* these are evident when the larva first escapes from the egg-membrane. Just as in the case of *Cyclops* so in *Apus* and other genera of Branchiopoda there comes a critical moult, at which antennae and mandibles are reduced to their adult proportions.

The Cladocera are remarkable for retaining throughout life the forked nature and swimming function of the second antenna, so that in their case the "critical" character of the moult is reduced to the loss of the distal joints of the mandible.

The Cirripedia also begin their free life as Nauplius larvae, which in all essentials, and even in such minute points as the many-jointed exopodite and feebly-jointed endopodite, agree with the larvae of *Cyclops*. They differ in the development of the dorsal integument into a great triangular shield, with two antero-dorsal horns, the **dorso-lateral spines**, and one postero-dorsal horn, the **caudal spine**, forming its three angles, and in the possession of a ventrally directed **pre-anal spine**. Further, we find in front of the first pair of appendages a pair of flexible antennae, the **frontal filaments**. As the larva grows the thoracico-abdominal portion of the body becomes divided into segments, on which six pairs of bilobed appendages are successively developed, whilst in the angle between this rudiment and the head the two pairs of maxillae appear as buds. Beneath the edges of the dorsal shield the rudiments of the paired eyes appear as dark areas. Then comes the critical moult, when the second pair of appendages completely disappears, and the third is reduced to

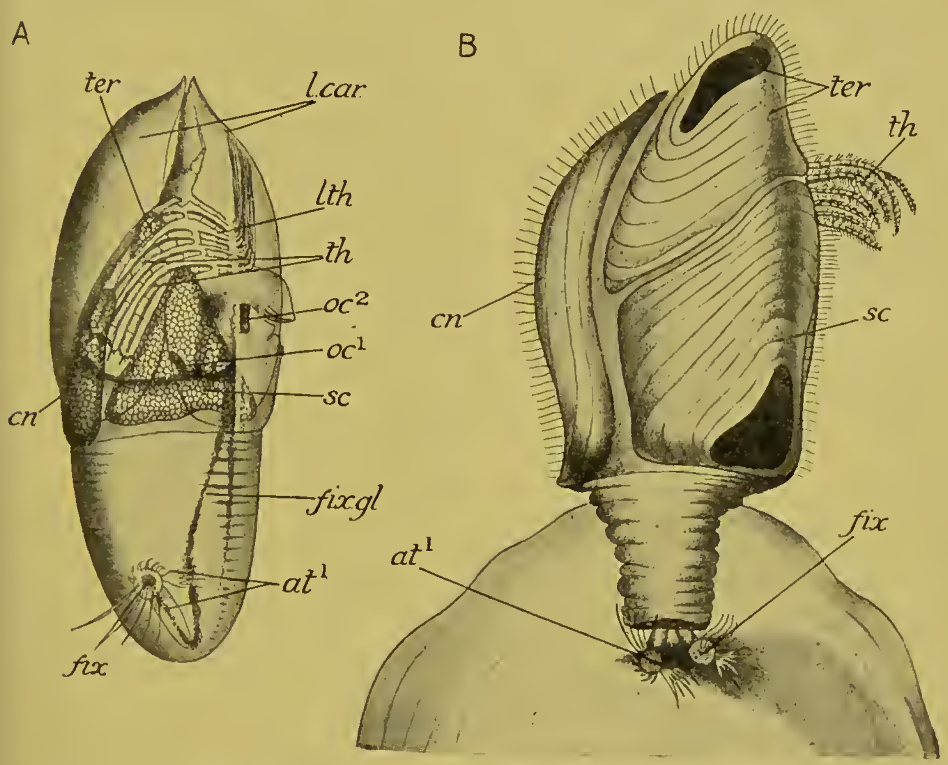


FIG. 148.—The fixation of the "Cypris" larva of *Lepas fascicularis*. (After Willemoes-Suhm.)

A, the larva just in the act of ecdysis after fixation. B, the young Barnacle fixed to a piece of dead shell. Letters as before. In addition, *cn*, carina; *l.car*, valves of larval carapace; *lth*, larval thoracic appendages being cast off; *sc*, scutum; *ter*, tergum; *th*, adult thoracic app.

its basal blade, whilst on the first antenna there is developed a disc for fixation. A bivalve carapace now appears which replaces the old three-cornered larval shield, the six pairs of thoracic legs acquire swimming-hairs and take over the swimming function, the compound eyes become functional, and the larva has now passed into what is termed the **Pupa** stage.

The pupa swims actively about for some time, but it takes no food. It finally settles on a suitable spot, and attaches itself by the disc on the first antenna in which a gland with a glutinous secretion opens; by the copious effusion of this secretion the larva is attached. An ecdysis now takes place and, by the preponderant growth of the skin of the ventral surface, the animal is rotated into a position in which it may be described as standing on its head. The praeoral part of the body grows very much in size and becomes the stalk, and shelly plates, the **scutum**, the **tergum**, and the **carina**, are secreted by the folds of skin which constitute the carapace; these calcareous plates replace the chitinous shields of the pupa. In this way the adult Barnacle condition is attained (Fig. 148).

In the development of the parasitic forms like *Sacculina*, which draw nourishment from the blood of their host through root-like extensions of the stalk, the Nauplius possesses no mouth and is fed by the yolk in its endoderm, and the pupa on fixation amputates the hinder part of the body.

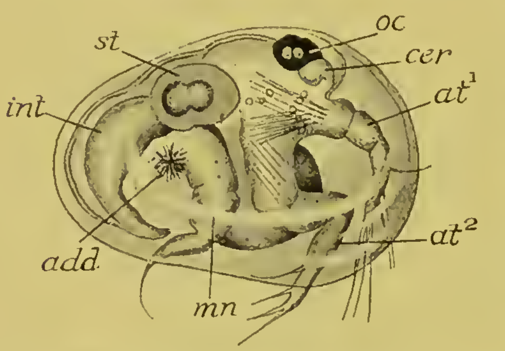


FIG. 149.—The Nauplius larva of *Cypris ovum*. (After Claus.)

add, adductor muscle of carapace; at^1, first antenna; at^2, second antenna; *cer*, cerebral ganglion; *int*, intestine; *mn*, mandible; *oc*, eye; *st*, stomodaeum (stomach).

The Ostracoda also enter on their free-life as creatures with the three appendages of the Nauplius, but the two flaps of skin constituting the bivalve carapace of the adult are already developed, and the second and third appendages consist of one branch only, the outer branch or exopodite being lost. The passage into the adult condition here is so gradual that one cannot speak of a critical moult. In the development of the carapace and the unforked character of the second and third appendages we have an anticipation of adult characters (Fig. 149).

When we turn our attention to the higher Crustacea we find that the Mysidacea among the Schizopoda and all the Cumacea carry the eggs in a brood pouch beneath the body of the mother, and from these eggs young Crustacea hatch out with all the essential features of the adult. But in the Euphausiadacea among the Schizopoda, and in some genera at least of the tribe Penaeidea among Decapoda, to which *Lucifer* belongs, the young leave the egg membrane as

Nauplii, showing, however, the ridge-like rudiments of two or three pairs of postoral appendages.

In the case of both *Euphausia* and *Penaeus* a succession of moults leads, as in other Nauplii, to the development of the thoracico-abdominal rudiment, and to the appearance in it of ring-like segments which first appear in the anterior or thoracic portion. The appendages belonging to these segments, which are the maxillipedes, are strongly developed, especially the first, which has a long exopodite used in swimming. A cephalic shield or carapace makes its appearance as a frill or fold round the head region, underneath which the future paired eyes appear as dark areas. In *Penaeus* the anterior part of this shield is produced into a sharp rostral spine.

The larva is now in the stage known as the **Protozoaea** (Fig. 150). Its further development into what is known as a **Zoaea** larva involves the growth and segmentation of the abdominal portion of the body, the thoracic segments remaining extremely narrow, especially the posterior ones, and the paired eyes become stalked. In Euphausiadacea the stalks are so short that the paired eyes do not project beyond the edge of the carapace, and the larva is consequently known as a **Calyptopis**. The majority of Decapoda pass through the Nauplius stage during their embryonic life and only enter on their larval life as Zoaeae.

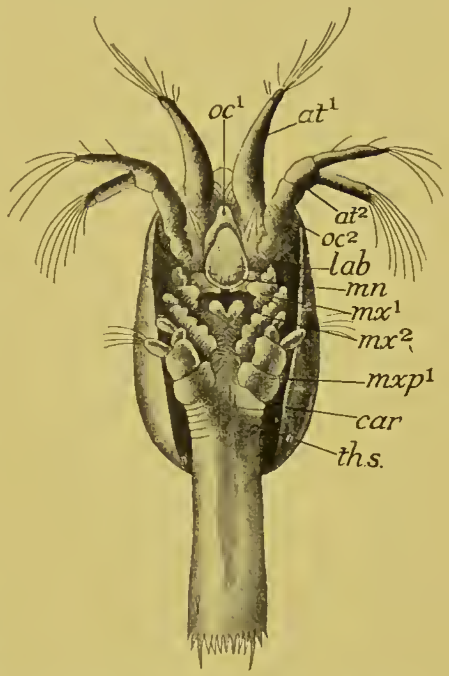


Fig. 150.—The Protozoaea larva of *Nyctiphanes australis*. (After Spence-Bate.)

Letters as in preceding figure. oc^1, simple anterior eye; oc^2, compound eye; *th.s*, thoracic segments.

ANCESTRAL CRUSTACEAN

We may now pause to consider how far the Nauplius larva may be regarded as representing an ancestral Crustacean form. Since it occurs in all the lower groups of Crustacea with no greater modifications than are found, for instance, in the different types of larva amongst Echinoidea; and since it also occurs in isolated cases amongst the higher Crustacea; and since, furthermore, an embryonic stage corresponding to the Nauplius is clearly marked in the development of every Crustacean egg which has been so far studied;

it is quite clear that the common ancestral stock of Crustacea passed through a larval stage corresponding to the Nauplius.

Now, on the principles laid down in the first chapter of this book, we are driven to conclude that the Nauplius represents a common ancestor of all Crustacea in however modified a form. Fritz Müller, in his work, *Für Darwin* (1864), concluded that all Crustacea were actually the descendants of a small oval, unsegmented species of animal with three pairs of legs. Hatschek (1877 and 1878), on the contrary, pointed out that such a conclusion implied that the Crustacea had no affinity with Annelida, nor with *Peripatus*, Arachnida, and Insecta, in all of which the early embryo was comparatively long and distinctly segmented, with a double series of cœlomic cavities. This conclusion Hatschek rightly thought to be incredible, and he therefore adopted the opposite opinion, namely, that the Nauplius had no ancestral significance at all, but that since in all Arthropoda—and Annelida, for that matter—the segments were developed from before backwards, so that the first was the oldest, there must in all exist a stage in which there were only three segments in the embryo, and, according to him, it was due entirely to a secondary modification that the eggs of some Crustacea were hatched when they reached this stage.

Korschelt and Heider, agreeing in the main with Hatschek, suggest that the Nauplius is an "Arthropodized Trochophore"—that it represents the Trochophore of Annelida with certain Arthropodan features precociously added. Balfour (1880), finally, whilst believing that the Nauplius in its present form was much modified, yet believed that it exhibited ancestral features, and that the hinder part of the body had formerly exhibited a segmentation which it had secondarily lost.

Amongst all the views we have recounted, that of Balfour seems to come nearest the truth. It was reserved for his pupil and successor, Sedgwick, to enunciate clearly what Balfour instinctively felt, viz. that the embryonic phase is secondarily developed out of the larval stage, and not *vice versa*. Indeed, Hatschek's view is thoroughly inconsistent with the fact that, when the larva does not hatch out as a Nauplius, a cuticle is produced and shed by the embryo whilst still within the egg-shell when it reaches the Nauplius stage, thereby showing that formerly this stage must have been passed through in the open, in the ancestors of the forms in which it is now purely embryonic.

We saw in Chapter I. that a larva, as compared with the actual ancestor which it represented, is usually greatly diminished in size, and that this diminution in size is not accompanied by a representation of all the organs which the ancestor possessed, also *diminished to scale*, because such diminution would render them ineffective; on the contrary, we saw that those organs which were functionally dominant in the ancestral condition are reproduced by the larva, while the others are suppressed.

We are, therefore, probably nearest the truth when we suppose that the Crustacean ancestor was like a Polychaete Annelid, with fairly numerous segments bearing somewhat feeble and membranous appendages like parapodia, but possessing greatly enlarged appendages attached to the first three segments, which fulfilled the major part of the work of locomotion, the first pair of these appendages alone having passed in front of the mouth. Corresponding to the diminution in size of the whole body in the larva as compared with its original size in the ancestor, the comparatively functionless posterior appendages have been suppressed.

But from such a form, with only one pair of appendages in front of the mouth, with the two next pairs in the form of powerful locomotor organs not specialised for either mastication or sensation, the Arachnida can be derived; and the fact that Onychophora and Insecta likewise have only one pair of antennae shows that they too could be traced back to such an ancestor. Finally, the extinct Trilobita, whose jaws bear long forked palps, and which possess only one pair of antennae, seem clearly to belong to the same cycle of affinity.

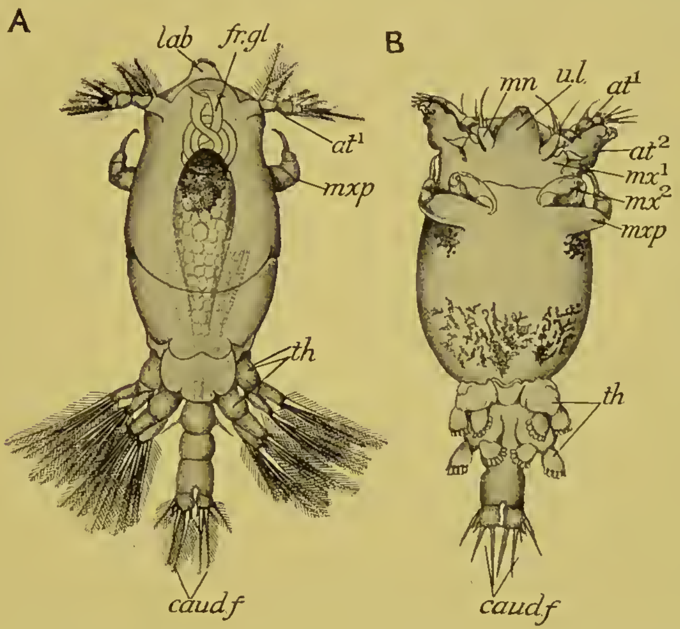


FIG. 151.—Dorsal and ventral views of the "Copepodid" larva of *Actheres ambloplitis*. (After Wilson.)

A, dorsal view. B, ventral view. *at^1*, first antenna; *at^2*, second antenna; *caud.f*, caudal fork; *fr.gl*, frontal gland; *lab*, labrum; *mn*, mandible; *mx^1*, first maxilla; *mx^2*, second maxilla; *mxp*, maxillipede; *th*, thoracic legs; *u.l*, under lip.

That this reasoning is justifiable and not far-fetched we may illustrate by taking a case where we may almost say that the ancestor is known, and where we are therefore in a position to compare the ancestor and its representation in the larva. This is the life-cycle of the parasitic Copepod, *Actheres ambloplitis*, which has been worked out by H. Wilson (1911). The adult lives on the gills of the rock-bass, *Ambloplites rupestris*: it is a sac-like organism fixed by two conjoined arms to the host; it shows no trace of Copepod structure except the long egg-tubes, which the female bears protruding from the end of her body. If we were to classify by adult structure alone, no one would dream of regarding *Actheres* as a Copepod; but yet every zoologist is fully convinced that *Actheres* is a modified Copepod—that is to say, that it is descended from an ancestor which was like *Cyclops* or *Calanus* or some other typical Copepod genus.

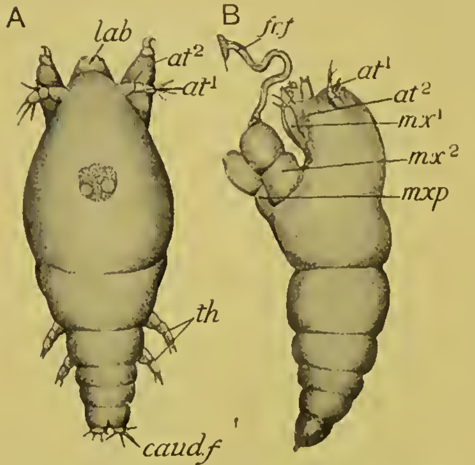


Fig. 152.—Dorsal and lateral views of just-fixed female of *Actheres ambloplitis*. (After Wilson.)

A, dorsal view. B, lateral view. Letters as in preceding figure; *fr.f*, frontal filament.

Now the Nauplius and Metanauplius stages are completed inside the egg membrane, and the young animal hatches out as what is termed a **Copepodid**—namely, in a form which every one would recognize at a glance as showing the typical structure of a Copepod, that is, of the ancestor. When, however, we look closely at this Copepodid larva we find that it differs from an ordinary Copepod in the following points:—(1) There are but two free segments in the thorax each carrying a pair of forked swimming appendages, whereas five such segments on the normal Copepod carry four pairs of forked swimming appendages and one rudimentary pair; (2) the exopodites and endopodites of these legs are not divided into joints, while the corresponding members in an ordinary Copepod are many-jointed; (3) the first antennae are short, stumpy, and few-jointed, as contrasted with those in an ordinary Copepod, where they are normally long and composed of many joints; (4) the second antennae in the Copepodid are likewise exceedingly short, and although forked each branch is unjointed and the inner one terminates in a hook, whereas in the normal Copepod this hook-like termination is not found; (5) in the jaws, *i.e.* mandible, maxillae, and maxillipede, there is nothing which could be described

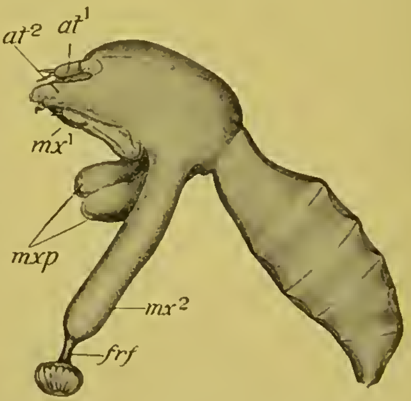


Fig. 153.—Lateral view of female *Actheres ambloplitis* after adult characteristics have been attained. (After Wilson.)

Letters as in preceding figures.

as atypical, but a long convoluted glandular tube lies in the mid-dorsal region of the head and opens at the front end of the carapace, and this tube secretes a long gelatinous filament.

The larval stage of the Copepodid only lasts thirty-six hours at most. The larva is swallowed by the rock-bass, and has the instinct to burrow into the mucous membrane of the pharynx of its host. When the pointed front end of the carapace of the parasite comes into contact with the bone of the gill arch of the host, the distal end of the filament already referred to is extruded from the frontal gland, and adheres thereto. The larva backs off, and the filament draws out; but before it is completely extruded the larva grasps the end of it by the incurved hooks of the second maxillae, and holds on.

A moult follows, in which the maxillipedes are shifted forward so that their bases are now situated between those of the second maxillae. The second maxillae are greatly enlarged, and have lost their segmentation though their two incurved ends still tightly grasp the filament. A sucking tube is formed by the union of a projecting labrum with an under lip, and inside this are the mandibles, though they can still be forced out through lateral slits between these lips. The minute first maxillae are attached to the sides of the sucking tube, and the two pairs of antennae are much shortened; they are reduced in fact to mere stumps (Fig. 154).

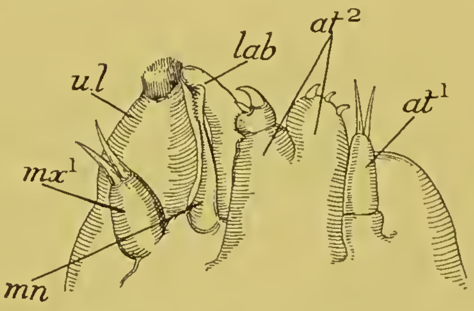


FIG. 154.—Enlarged view of gnathites and lips of female *Actheres ambloplitis* seen from the side. (After Wilson.)

Letters as in preceding figures. In addition, *u.l*, under lip.

The animal can now stab the vascular gill of its host with its needle-like mandibles and suck its blood, and it grows rapidly in size, moulting frequently. At the first of these moults practically the adult form is attained, all trace of the thoracic appendages is lost, and the maxillipedes are transformed into blunt shapeless lobes. The filament of the female shortens till the claws of the second maxilla are actually in contact with the skin of the host; that of the male, however, remains long, and he appears to crawl around like a tethered goat until he finds a female; then he relaxes his hold on the filament and seizes the female with his claws, and so is in a position to effect sexual union.

An examination of the differences between the Copepodid larva and the typical Copepod shows examples of all the changes we postulated in explaining the Nauplius;—we have the reduction in segmentation, and the disappearance of appendages, or rather the replacement of a homologous series of appendages by a smaller number of similar ones; in fact there is a functional rather than a proper morphological representation of these in the Copepodid larva. If, then, it be a sound principle of science to reason from the known to the unknown, we are justified in regarding the differences between the known ancestor of *Actheres* and the larva by which it is represented, as a means to deduce the unknown ancestor of all Crustacea from the Nauplius larva, by which we believe that ancestor to be represented.

A similar problem confronts us when we consider the significance of the typical larva of the higher Crustacea, the Zoaea. We have already seen that this larval form develops out of the Nauplius larva in the Euphausiadacea amongst Schizopoda, and the Penaeidea amongst Decapoda. But all the Anomura and Brachyura, so far as is known, begin their free-swimming life with the Zoaea stage, and amongst the Macrura this is true of the Caridea.

All Zoaea larvae agree in possessing (1) paired stalked compound eyes, (2) a carapace which overlaps and conceals part of the thorax, and (3) a segmented abdomen. All agree further in possessing (4) three pairs of limbs (*i.e.* mandibles, and first and second maxillae) which are thoroughly modified to form jaws, (5) in having the first few segments of the thorax well developed, and carrying forked limbs termed maxillipedes on which a large part of the locomotor function falls; and finally they agree (6) in having the hinder segments of the thorax either entirely suppressed or very thin, and without appendages or with mere rudiments of appendages.

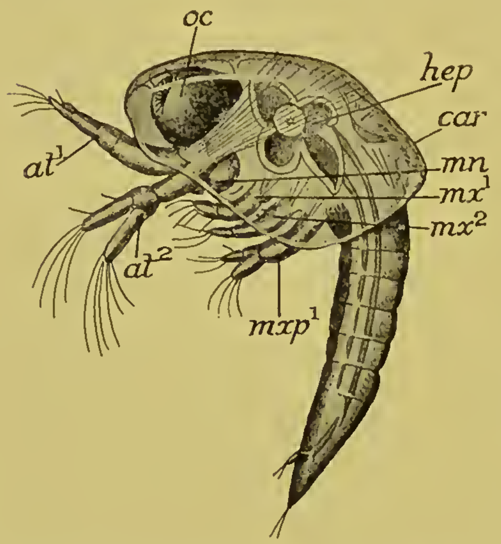


FIG. 155.—"Calyptopis" Zoaea of *Nyctiphanes australis*, lateral view. (After Spence-Bate.) *car*, carapace; *hep*, liver lobules; *oc*, compound eye.

But whilst the general features of the Zoaea may be regarded as constant, its specific features vary from group to group. The Zoaea of the Euphausiadacea and of the Penaeidea is characterized by retaining a large forked second antenna like that of the Nauplius, which assists in swimming.

The Zoaea of Euphausiadacea has one pair only of maxillipedes well developed, and it has several other well-marked peculiarities: thus the last pair of abdominal appendages is developed, and the border of the carapace projects considerably and conceals the short eye-stalks from view when the larva is seen from above, hence, as already mentioned, it is termed **Calyptopis** (Fig. 155).

The Zoaea of the tribe Penaeidea, amongst Decapoda, differ from the **Calyptopis** in having two pairs of maxillipedes developed, and a rudiment of the third, and in having small rudiments of appendages even on the thin posterior thoracic segments; also in having a median rostral and two postero-lateral spines on the carapace, and in having long-stalked compound eyes.

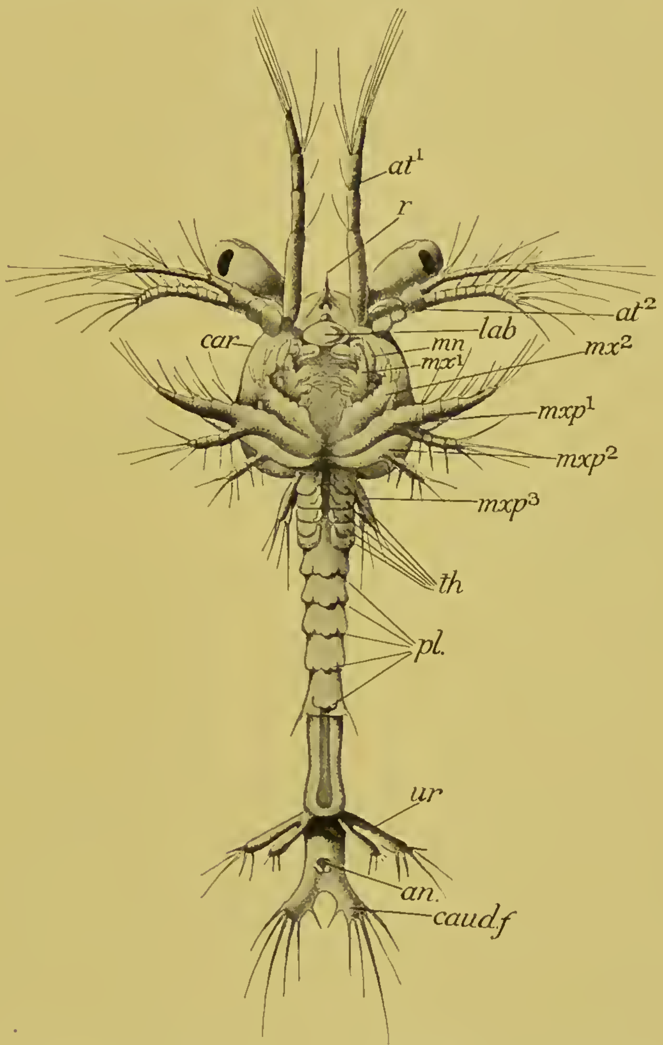


FIG. 156.—Zoaea of *Penaeus*, ventral view. (After Claus.)

mxp^{1-3}, first, second, and third maxillipedes ; *pl*, rudiments of pleopods (*i.e.* abdominal appendages) ; *r*, rostrum ; *th*, rudiments of hinder appendages of thorax ; *ur*, uropods (last abdominal appendages).

The Zoaea of the shrimps and prawns (*i.e.* Caridea) agrees with the Zoaea larva of the Penaeidea in possessing these spines on the carapace, but it differs from them and agrees with the Zoaea of the higher Decapoda in two respects; (1) in having the exopodite of the second antenna converted into a flat unjointed scale (**squame**), as in the adult Decapod, (2) in having the endopodite of this appendage much shortened so that this limb is no longer locomotor in function.

Three pairs of maxillipedes are, however, developed, and on them consequently devolves the whole function of locomotion. The hinder segments of the thorax are completely suppressed, and no trace of appendages is found on the abdomen (Fig. 157).

The Zoaea of the Anomura is very similar in general appearance to the Zoaea of Caridea, but it only possesses two pairs of maxillipedes, and, generally speaking, no trace of the hinder thoracic appendages is present at birth although they appear after the first moult, and the rostral spine is always very long and sometimes enormously elongated. Finally, the Zoaea of the Brachyura, while agreeing in most points with the Zoaea of the Anomura, differs from it and all other Zoaeas in possessing a long mid-dorsal spine sloping backwards.

The very same controversy which developed concerning the meaning of the Nauplius, raged over the significance of the Zoaea. Some, like Dohm (1870), held it to represent an ancestor of the Schizopoda and Decapoda; others, like Claus (1878), pointed out that such a conclusion would imply that in the ancestral Decapod the hinder part of the thoracic region was unsegmented, and that these segments, when they did appear, must have been secondarily intercalated. If such reasoning were justified it would sever the higher Crustacea from all connection with the lower Crustacea, for in these latter the segments follow one another in development in uninterrupted series from before backwards; and so the conclusion was drawn that the Zoaea had no significance whatever.

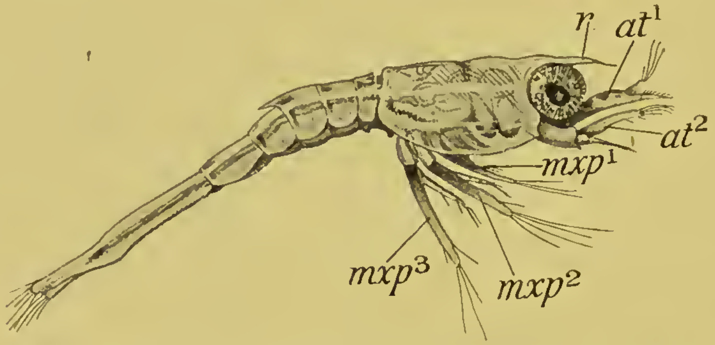


FIG. 157.—Zoaea larva of *Crangon vulgaris*, lateral view. (After Sars.) Letters as in previous figure.

Balfour then pointed out that in the more primitive types of Zoaea the posterior thoracic segments, although very thin, are distinctly present, and therefore surmised that the Zoaea might represent in a modified form the ancestor of higher Crustacea. On the principles laid down in this book, we must agree with him. The Zoaea was clearly a larval form in the life-history of an ancestor common to the Schizopoda and Decapoda, in a word, we might say, to the primitive Malacostracan, and therefore represented an ancestor of this Malacostracan. But in that case, what stage in the evolution of the polychaete worm into the shrimp does the Zoaea represent? Obviously one in which (1) the first antenna had become purely sensory, (2) the second antenna had moved in front of the mouth and had lost its masticatory function, (3) the mandible had become purely masticatory, (4) the next two pairs of appendages (maxillae) had been modified into jaws, (5) the main swimming function had been thrown on the first two or three appendages of the thorax, and, (6) compound eyes and a carapace had been developed.

If we read over this list we might conclude that the Phyllopod genus *Apus*, if it had possessed better developed antennae, would have given us a good idea of what the ancestor represented by the Zoaea looked like. The first two pairs of thoracic appendages of *Apus* are developed into long antenna-like organs. All the others, of which there is a great number, are thin and parapodia-like. These, which must have existed in the ancestor, are not represented in the Zoaea, owing doubtless to their physiological unimportance and the diminution in size of the larva. We cannot, however, well imagine that the abdomen in the ancestor was devoid of appendages, although it is so in most Zoaeae. In the Zoaeae of the Penaeidea, indeed, the appendages of the last segment are developed, and there are vestiges of appendages on the other segments of the abdomen.

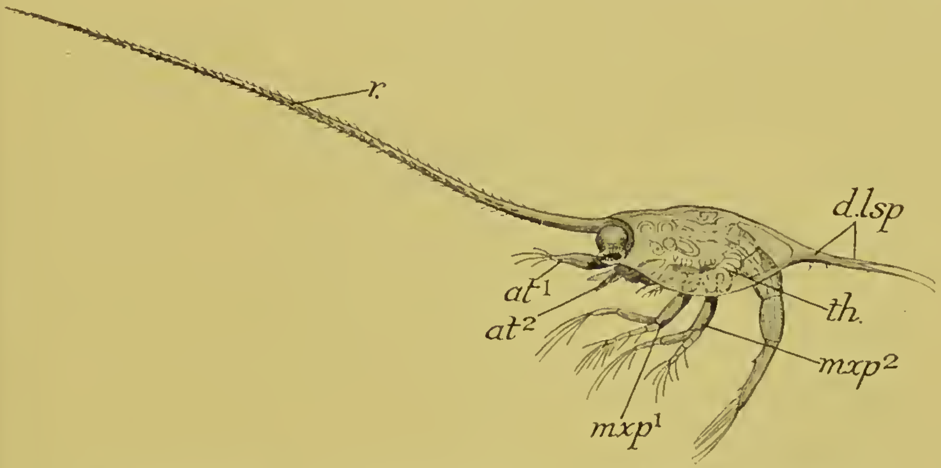


FIG. 158.—Zoaea larva of *Porcellane longicornia*, after the first moult. (After Sars.) *d.l.sp*, dorso-lateral spines of the carapace; *r*, enormously elongated rostral spine; *th*, rudiments of hinder thoracic appendages.

The abdomen, as a region with peculiar appendages, is characteristic of the Malacostraca, and the physiological necessity which led to its evolution can be inferred by watching the way in which it is used by Zoaeae. Many of these swim on their backs, using the long spines which project from the carapace as a keel. The maxillipedes are used as oars, and *the abdomen functions as a rudder.*

We can now form to ourselves a picture of the course which evolution followed in transforming the ancestor represented by a

Nauplius into a primitive Crustacean. The second antenna became gradually shifted forward and lost its masticatory function, while the third appendage became exclusively masticatory and its distal joints shrank to an insignificant palp. At this stage of evolution the animal was assisted in mastication by the modification of the appendages of the next two or three segments, which formed maxillae, but which were never greatly changed from their original parapodia-like condition.

When these changes had been effected the ancestor was definitely a Crustacean, and from this level the Ostracoda may well have branched off. In the Ostracod the number of pairs of maxillae varies from one to three in different genera, and what in one genus is a maxilla in a neighbouring genus may be a small thoracic limb. Swimming is mainly performed by the antennae. Finally, in this group alone among Crustacea, there are retained throughout life two pairs of excretory organs, viz., a pair of antennary glands as in the higher Crustacea, and a pair of maxillary "shell"-glands as in the lower Crustacea.

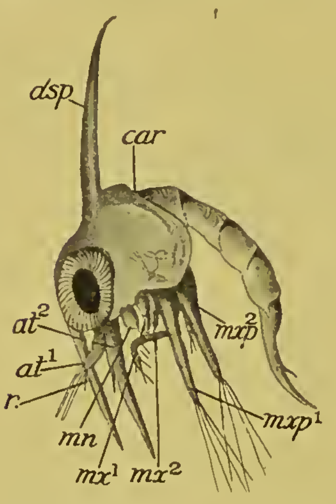


FIG. 159.—Zoaea larva of the Crab *Xantho*. (After Cano.)

d.sp, median dorsal spine of the carapace; *r*, rostrum of carapace deflexed and acting as a frontal spine.

The Cladocera also must have branched off about the same period of evolution from the common stock, and this is true also of some of the Phyllopoda. Those genera, however, like *Apus* and *Branchipus*, in which the antennae have lost their swimming function, represent the higher stage of development.

Following the stage of evolution which we have been discussing, a new stage supervenes in which the swimming function began to be *handed on* to the first thoracic appendages, while the hinder part of the body became specialized to form a rudder by the diminution in size of its appendages.

The Copepoda and Cirripedia seemed to have diverged at this point. In them as in the Zoaea larva the appendages of the hinder segments are suppressed altogether—a phenomenon doubtless due to the diminution in size, which affects these Crustacea in the same way as it affects the larvae. A condition just previous to this stage is also represented by the Zoaea of Penaeidea and Euphausiadacea, in which a large portion of the swimming function is still carried out by the second antenna. But the process of "handing on" the swimming function to the thoracic appendages, once initiated became progressive, and soon the second antenna became relieved entirely of its swimming functions, which were then exclusively performed by the thoracic appendages, whilst the second antenna was set free for sensory functions.

This stage is represented by the Zoaea larvae of Caridea, Anomura, and Brachyura. The Zoaea is transformed by several moults; first into a Metazoaea, in which the rudiments of the appendages of the abdomen and of the hinder segments of the thorax appear; secondly into a so-called "**Mysis**" stage, in which, typically, the hinder segments of the thorax bear forked limbs designed to assist in swimming, and in which the first appendages of the thorax tend to become somewhat diminished in size and degraded from the rank of locomotor organs of prime importance to that of maxillipedes.

This Mysis larva, as its name implies, is of such obvious ancestral significance that no one has ever attempted to deny that it represents a Schizopod ancestor. We can, however, even here trace the work of the same modifying tendencies which have obscured the ancestral significance of the Nauplius and the Zoaea. In such of the Nephropsidea as do not complete their development within the egg-shell (cf. *Homarus,* the lobster, and *Nephrops,* the Norway lobster) the larva emerges in the Mysis condition, with this difference, that the abdominal appendages are at first quite suppressed.

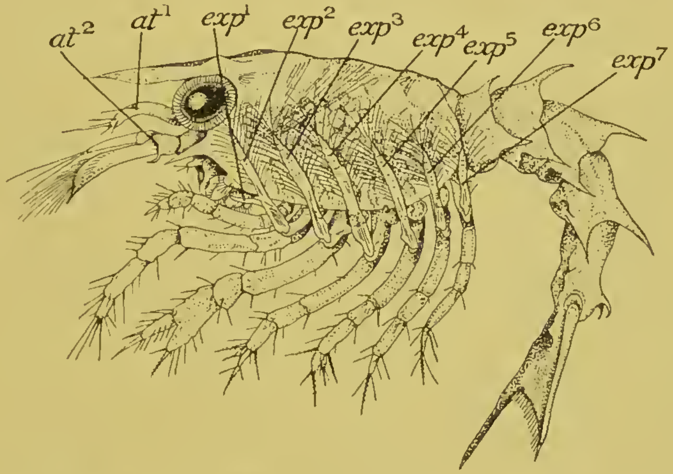


FIG. 160.—"Mysis" larva of *Homarus americanus,* lateral view. (After Herrick.) ex^1-ex^7, the seven exopodites borne by seven of the thoracic legs.

In the Loricata, of which the rock-crayfish, *Palinurus,* and the square-nosed lobster *Scyllarus* are the best known representatives, the larva emerges from the egg-shell in a singularly modified Mysis stage (Fig. 161). In this larva the thorax is broad and flat and of a glassy transparency; the abdomen, though distinctly divided into segments, is very small and has no rudiments of appendages. The thorax has only six of the eight pairs of appendages which it should possess if normally developed, and of these, those representing the first two pairs of maxillipedes are small but those representing the third maxillipede and the first three pairs of walking thoracic legs, have enormously long endopodites and short outer forks (exopodites).

Here we see, as has been repeatedly emphasized in this book, that Nature treats as a single organ an apparatus consisting of several pairs of metamerically arranged organs, which co-operate in the performance of a single function; and when the whole organ is diminished in size—as when it is reproduced in a larva—the number of component similar organs is reduced also.

This modified Mysis is known as a **Phyllosoma** or glass-crab. As it grows and moults the first maxillipede grows larger, and the last two pairs of thoracic legs also make their appearance; so also do the appendages of the abdomen, and thus the adult condition is approached. The explanation of the singular appearance of this larva is, that in the case of the Loricata the Mysis has ceased to be an actively-swimming organism and has become a surface-drifter; the long legs being widely spread out and acting as supports.

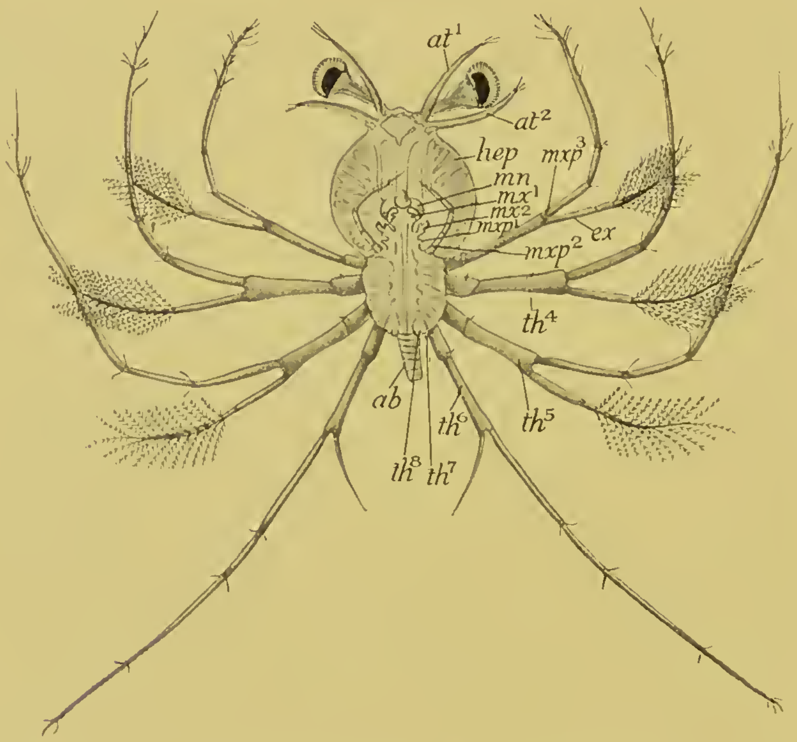


FIG. 161.—"Phyllosoma" larva of *Palinurus vulgaris*, ventral view. (After Cunningham.)

ab, abdomen; *hep*, liver saccules seen through *ex*, exopodite of thoracic appendages.

Another series of modifications of the Mysis stage have been described by Sars (1891) in the case of the Crangonidae amongst Caridea. The Zoaea of these forms it will be remembered has, when hatched, *three* pairs of maxillipedes developed as forked swimming appendages. In subsequent moults a varying number, but in no case do all the remaining segments of the thorax develop swimming appendages. Thus in *Crangon* only one extra segment develops forked appendages (Fig. 162), in *Cheraphilus* two segments develop appendages with exopodites, in *Pontophilus* two, and in *Sabinea* one only. Those segments that do not develop swimming appendages give rise to simple, unforked appendages, which at the last moult develop directly into the hinder walking legs of the adult, as in the case of the Loricata, and we may add, as in the case of the Mysis larva which develops out of the Zoaea larva of Thalassinidae, another family of the Caridea.

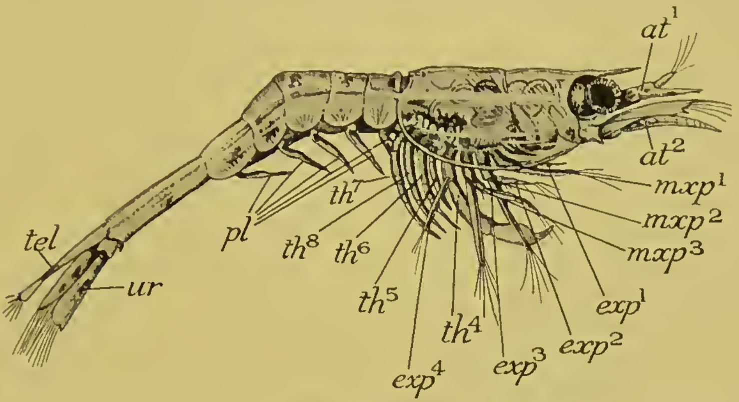


Fig. 162.—"Mysis" larva of *Crangon Allmanni*, lateral view. (After Sars.)

tel, telson; exp^{1-4}, the four exopodites borne by the three maxillipedes and great chela respectively; *pl*, pleopods (abdominal appendages); *ur*, uropods (last abdominal appendage); th^{4-8}, the appendages which will form the five pairs of walking legs.

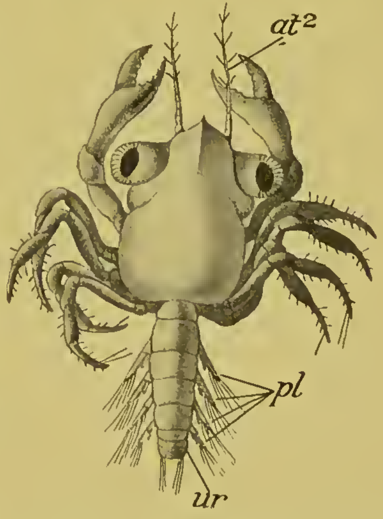


Fig. 163.—"Megalopa" larva of the Crab *Pilumnus*, dorsal view. (After Cano.)

Letters as in preceding figures.

Finally, in the Brachyura *all* the thoracic segments behind the first two which bear the swimming appendages of the Zoaea, develop only rudimentary bud-like appendages whilst the larval swimming stage persists, but when this is over and the larva begins life at the bottom, then these appendages develop directly into the walking legs without ever passing through a forked stage.

Thus, in the life-history of the crabs the Mysis stage has been completely eliminated, but nevertheless the Zoaea does not change into the adult stage but into a form called a **Megalopa,** in which the carapace is longer than broad, and in which all the segments of the abdomen possess well-developed swimming appendages. This larva obviously represents a Macruran stage in the ancestry of crabs, just as clearly as the Mysis larva represents a Schizopod stage in the ancestry of Macrura. The Megalopa is transformed into the adult by one or two moults.

The life-history of the Anomura closely resembles that of the Brachyura; in their case also the Mysis stage is omitted, but the third appendage of the thorax, the third maxillipede, becomes functional before the critical moult which ends the free-swimming life. The post-larval stage of the Paguridae or hermit crabs, which corresponds to the Megalopa stage of Brachyura, is distinguished by the possession of a symmetrically-developed abdomen—an indication that these asymmetrical forms are descended from ancestors which were bilaterally symmetrical. It has been found that the abdomen of the young Pagurid becomes asymmetrical *before* the animal seizes on an empty shell in which to shelter its abdomen. This fact is of extraordinary interest on account of its bearing on the nature of heredity.

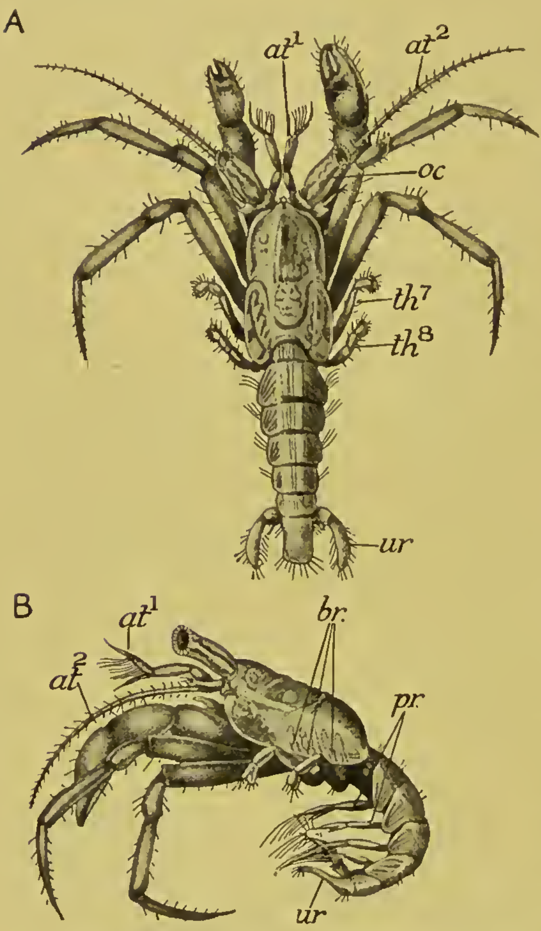


Fig. 164.—Two views of first post-larval stage of *Eupagurus bernhardi* corresponding to the Megalopa stage of Brachyura. (After Sars.)

A, dorsal view. B, lateral view. *br*, branchiae attached to thoracic limbs seen through the carapace; th^7, th^8, the two last pairs of thoracic limbs, reduced in size.

The curious groups of Stomatopoda, which agree with Schizopoda in having only three pairs of appendages modified as jaws, and in having exopodites on some of the thoracic appendages; but which differ from them, and indeed from all other Malacostraca, in having the first five pairs of thoracic appendages modified into grasping claws, and in having gills developed on the abdominal appendages, present a life-history which affords further confirmation of the laws of larval modification, laws which have made themselves clear in the course of our study. There is some reason to believe that the life-history of some Stomatopoda begins with a Nauplius larva. At any rate Lister (1898) has captured in the open sea a Metanauplius in which the mandible is reduced to its blade, and in which the rudiments of the maxillae are developed. This larva is shown to belong to a Stomatopod by the fact that it possesses already two stalked compound eyes, which distinguish it from the Metanauplii of all other groups of Crustacea. It has a triangular carapace resembling that of the Zoaea larva, and the hinder part of the body is formed of an unsegmented abdomen terminating in a jointed caudal fork.

The next stage in the life-history which is known is the **Erichthoidina** stage, which Balfour compared to a Zoaea larva. In this stage the larva has a precisely similar carapace and stalked eyes, but the first antenna has developed a second flagellum and so become forked, whilst the second antenna is now unforked. There are the usual three pairs of jaws, and these are followed by no less than five pairs of forked swimming appendages. The abdomen is, however, almost unsegmented, only the *first* segment with rudiments of its appendages being present, and it ends like the abdomen of most Zoaeae in a broad tail-fan. Thus the larva is more like a Mysis larva than a Zoaea, but differs from both these types of larvae in the character of the abdomen.

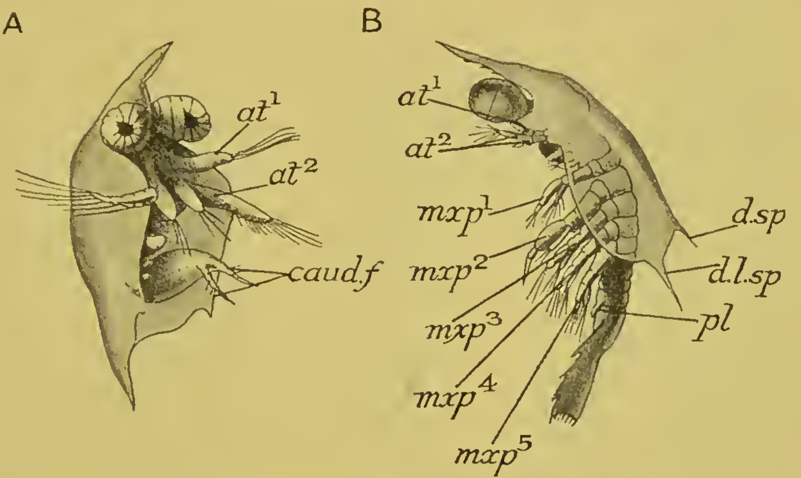


FIG. 165.—Two stages in the development of a Stomatopod larva. A. After Lister. B. After Hansen (from Lister).

A, Early "Metanauplius" stage. B, So-called "Zoaea" or Erichthoidina stage. *d.sp*, dorsal spine; *d.l.sp*, dorso-lateral spines; mxp^{1-5}, the five maxillipedes; *pl*, the rudiment of the appendage of the first segment of the abdomen.

In subsequent moults the abdomen becomes segmented and develops its appendages, whilst the endopodite of the second appendage of the thorax develops into a great hooked claw, and the first appendage develops into a long slender unforked limb, the hinder three pairs of appendages dwindle into insignificant rudiments or vanish altogether. In this way the **Erichthus** stage is reached, which is sometimes termed a **Pseudozoaea** because it has only the first two pairs of thoracic appendages well developed, the next three being represented by mere stumps, and the appendages of the hindermost thoracic appendages being totally absent. This is transformed into the adult by a series of moults in which the diminished or vanished appendages reappear in the form of grasping claws, and in which the hindermost segments of the thorax develop their appendages as long legs with the rudiments of exopodites.

In the case of other Stomatopoda the embryo develops within the egg until it has reached the Pseudozoaea stage; it then emerges as an **Alima** larva, which differs only in unimportant details from the Pseudozoaea. The subsequent development of the Pseudozoaea is the same as that of the larva.

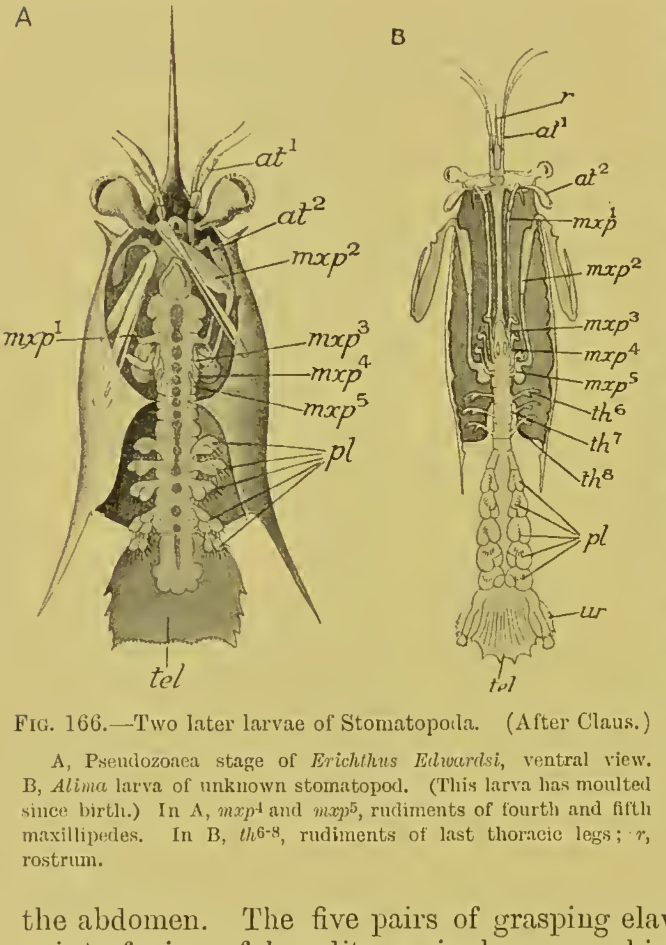


FIG. 166.—Two later larvae of Stomatopoda. (After Claus.)

A, Pseudozoaea stage of *Erichthus Edwardsi*, ventral view. B, *Alima* larva of unknown stomatopod. (This larva has moulted since birth.) In A, *mxp*^{4} and *mxp*^{5}, rudiments of fourth and fifth maxillipedes. In B, *th*$^{6-8}$, rudiments of last thoracic legs; *r*, rostrum.

Now these life-histories justify us in regarding the Stomatopoda as derived from Schizopod ancestors, in which the anterior thoracic appendages were gradually converted into grasping claws whilst the swimming function was thrown on the abdominal appendages; just as we believe that Decapoda are derived from Schizopoda, in which the posterior thoracic segments were developed into ambulatory legs whilst the swimming function is equally relegated to the abdomen. The five pairs of grasping claws constitute, from the point of view of heredity, a single organ which is reproduced in the Pseudozoaea and Alima larvae as two large functional pairs only.

The Sessile-eyed Decapoda, including Cumacea, Anisopoda, Amphipoda, and Isopoda, and the division Mysidacea of Schizopoda, which are all grouped by Calman (1909) under the name Peracarida, enter on their free life similar to their parents in all essential features; but the just-born young of all these groups agree in having the last segment of the thorax and its appendages suppressed; another, if less well-marked, example of the same rule as that exemplified by the larvae of Stomatopoda.

Amongst the Isopoda, however, there are a considerable number of genera which have developed suctorial mouths by a union of upper and under lips, and which become parasitic on other Crustacea. The shapes of some of these parasitic forms have become distorted out of all recognition, especially in the female sex. In *Portunion* (Fig. 168), for instance, which belongs to the family of the Entoniscidae, the "oostegites" of the thoracic legs become enlarged into long leaf-like folds which are packed together like the leaves in a bud, the legs themselves having completely disappeared. The head, with its sucking apparatus, forms a small rounded knob, whilst the abdomen is bent back over (not under) the thorax, and its appendages take the form of crimped laminae.

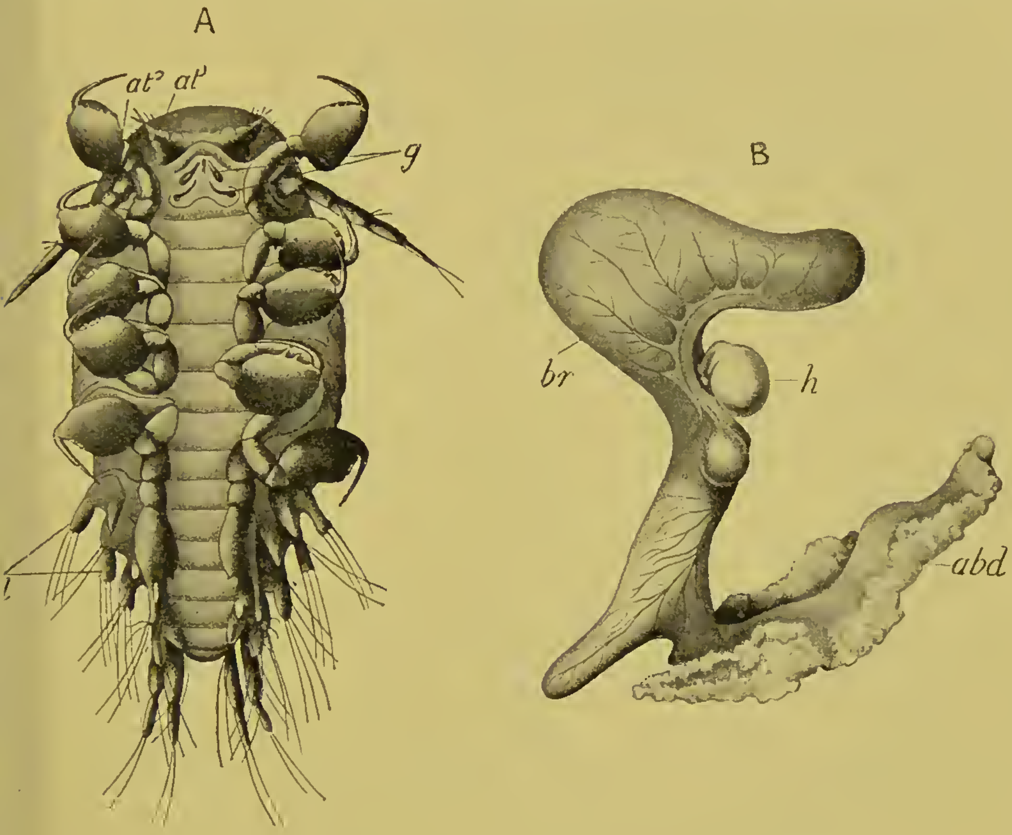


Fig. 167.—Larva and adult female of *Portunion maenadis*. (After Giard.)

A, larva, dorsal view. B, adult female, lateral view. *abd*, abdomen; *at*^{1}, antennule; *at*^{2}, antenna; *br*, brood-sac composed of conjoined ovigerous plates of thorax; *g*, jaws or gnathites; *h*, head; *pl*, swimmerets or pleopods.

The larvae of these extraordinary forms have the depressed body and segmentation of a normal Isopod (Fig. 167): a short pair of first and a long pair of second antennae, and six pairs of unforked thoracic legs followed by six pairs of forked abdominal ones. The jaws are already lancet-like and the lips united. In all but these last two points they resemble the young of normal Isopoda when they leave the brood-pouch, and not even the most determined opponent of the recapitulation theory could deny their ancestral significance.

It is a tacit assumption of the recapitulation theory that advance in evolution is correlated with a change in the environment, and with the consequent acquisition of new kinds of food by the animal in the adolescent stage of its life-history. The evidence that this theory is well founded comes out more and more strongly the more the embryology of the various members of the animal kingdom is studied; but it is exceedingly difficult to reconcile it with modern work on the subject of heredity, which appears to point to the conclusion that changes in morphology are due to changes occurring in the nuclear matter of the germ cells before fertilization. When such chemical changes have been effected—why, it may be asked, should their influence appear just at that moment of development when the environment is changed? Light might be thrown on this question by a careful and systematic study of the life-histories of parasitic and abnormal forms, belonging to large families or orders in the animal kingdom, which show well-established and stereotyped normal features. Work on these questions will form one of the most fascinating features of future embryological study.

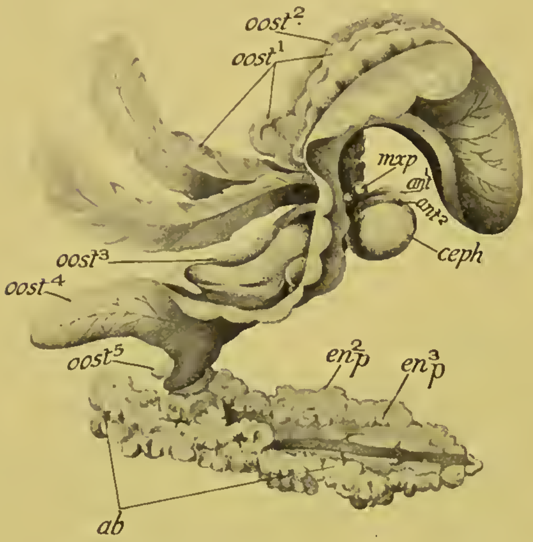


FIG. 168.—Adult female of *Portunion maenadis*, with appendages dissected out. (After Giard.)

ab, abdomen; *ant*^{1}, vestige of first antenna; *ant*^{2}, vestige of second antenna; *ceph*, swollen head; *mxp*, rudiment of maxillipede; *enp*^{2}, the endopodite of the second abdominal appendage; *enp*^{3}, the endopodite of the third abdominal appendage; *oost*^{1}, the three lobes of the first oostegite of the right side; *oost*^{2}, the second oostegite on the right side; *oost*^{3}, the third oostegite on the right side; *oost*^{4}, the fourth oostegite on the right side; *oost*^{5}, the fifth oostegite on the right side.

ARACHNIDA

Classification adopted—

Delobranchiata	**Xiphosura**
Embolobranchiata	**Scorpionidea** **Pedipalpi** **Araneina** **Acarina**
Pentastomida (incertae sedes)	

The most primitive Arachnid living is undoubtedly the horse-shoe crab *Limulus*, the development of which has been studied by many authors, but most recently by Kingsley (1892, 1893) and Kishinouye (1893). The distribution of this genus renders it unsuitable as a type of the Arachnida, for it is practically inaccessible to European students. A similar objection applies to the Scorpion which, on the whole, must be regarded as the most primitive of existing land Arachnids; its absence from the temperate regions of both hemispheres is a serious drawback, and hence we select our type for special description from amongst the ubiquitous spiders, and take as our chief authority Kishinouye (1891–94). This observer has not only published the most thorough work on the subject but has examined the course of development in different genera belonging to different families, and found it identical in all important points.

AGELENA

Amongst the types described by Kishinouye there is one *Agelena*, a cellar spider, representative species of which are found all over the northern hemisphere, and one of which, *A. labyrinthica*, formed the subject of an embryological research by the late Prof. Balfour. Quite recently another author, Kautsch (1909, 1910), has also studied the development of *Agelena labyrinthica*. His conclusions in the main confirm those of Kishinouye, but in some points he has penetrated further in the analysis of the development of this species than Kishinouye; in other points, again, it seems likely that his variations from Kishinouye's account will turn out to be incorrect. We shall therefore select *Agelena* as a type. The species of this genus can be kept and will breed in captivity. The eggs of *Agelena*, as of all spiders, are enclosed in a cocoon of silk; the mother attaches this cocoon to a corner of the cage, and in this way eggs of all stages of development can be obtained.

Kishinouye had also recourse to a "wolf spider," *Lycosa*, which spins no web but wanders about in search of prey, and species of this genus are as widely distributed as those of *Agelena*. Since *Lycosa* carries about its eggs in a cocoon attached to the underside of its abdomen, where they remain till the young spiders are hatched, the later stages in development can always be obtained by capturing the adult female; but *Lycosa* cannot be induced to breed in captivity.

Kishinouye's method of dealing with the eggs is as follows. The earlier stages were plunged into water of a temperature of from 70° to 80° C., the later stages were placed in cold water which was gradually heated to this temperature. When the eggs had become opaque and white they were removed, and when cool they were placed at once in 70 per cent alcohol. After 24 hours in this reagent, they were examined under the dissecting microscope and the egg membrane pricked with a needle, the hardening was then completed in ascending grades of alcohol. It was found that after staining in picro-carmine the paraffin penetrated better than after other stains. It is clear that the celloidin-paraffin method of embedding would be more suitable than that of pure paraffin, which Kishinouye employed.

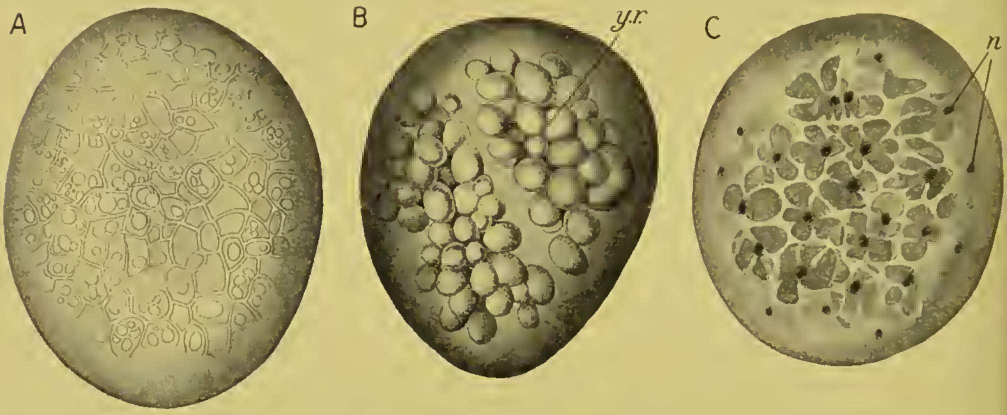


Fig. 169.—Three stages in the segmentation of the egg of *Agelena*. (After Kishinouye.)

A, stage before the division of the zygote nucleus has taken place, showing the ends of the radiating columns of the yolk; B, stage in which the zygote nucleus has divided once; C, stage in which the zygote nucleus has given rise by repeated divisions to a considerable number of nuclei. *n*, nuclei surrounded by islands of cytoplasm; *y.r*, yolk rosette.

The egg possesses an inner thin vitelline membrane and an outer chorion, and the surface of the latter is beset by a mosaic of very fine granules. At the centre of the egg there is an accumulation of protoplasm, which contains the zygote nucleus, and is termed by Kishinouye the **centroplasm**. There is also, as in all centrolecithal eggs, an outer rind of protoplasm, which Kishinouye terms the **periplasm** (*per*, Fig. 11). Between the centroplasm and periplasm the yolk is arranged in a series of radiating columns, each column consisting of a radiating series of short tangential rows of yolk granules. Between adjacent columns Kishinouye surmises the existence of radiating sheets of protoplasm connecting the centroplasm with the periplasm, but he was not able certainly to demonstrate their existence.

The periplasm is marked out into polygonal areas which correspond to the yolk columns, and indeed form caps to them. These areas cause the egg, when it is newly laid, to appear as if it had already undergone segmentation, and the whole structure of the egg at this time irresistibly reminds one of the segmented egg of *Astacus* with its primary yolk pyramids. Nevertheless, the resemblance between the two eggs is purely illusory; for the egg of the spider when newly laid contains only one nucleus, and the polygonal areas seen in the periplasm are due to the pressing of the soft periplasm against the resisting yolk columns as the egg is squeezed in passing down through the oviduct.

When the nucleus divides into two the yolk columns become massed together into two rosette-like bundles, with one nucleus in the centre of each bundle; and a small blastocoele appears at the centre of the egg. The same process is repeated when the next division takes place, and so there are as many yolk rosettes as nuclei. In consequence of this rearrangement of the yolk columns they shift with relation to the polygonal areas of the periplasm, and these latter no longer form caps to them. As the nuclei increase in number the segmentation cavity enlarges, and the nuclei gradually travel towards the surface of the egg. When the number of nuclei has attained 30, all reach the surface; but the multiplication of the nuclei still continues, and when 100 nuclei have been formed the periplasm becomes completely separated from the yolk and transformed into a blastoderm of rounded cells.

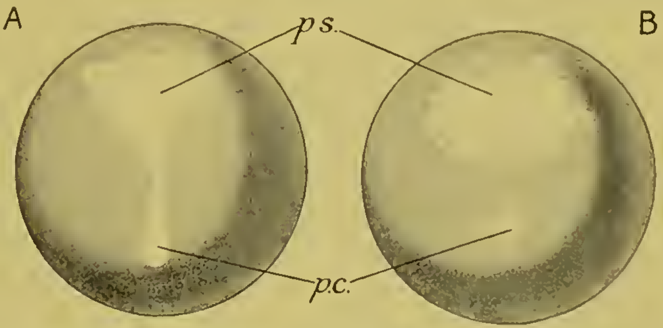


FIG. 170.—Surface views of the developing egg of *Agelena labyrinthica*, showing the primitive streak and the primitive cumulus. (After Kautsch.)

A, stage in which the primitive streak and the primitive cumulus are connected. B, stage in which the connection between the two is broken and the primitive cumulus is migrating backwards (?). (After Kautsch.)

The blastocoele seems by this time to have disappeared. As the blastodermic cells multiply they become more flattened, except at one spot, which Kishinouye names the **primitive ventral thickening** and which we may term the **primitive streak.** Here the blastoderm cells remain rounded; they proliferate cells into the interior of the egg, and thus initiate the gastrula stage and the formation of layers. The primitive streak becomes elongated, and at one end of it another thickening appears, caused also by the cells of the blastoderm retaining a rounded shape and proliferating inwards.

What the meaning of this secondary thickening is Kishinouye could not ascertain, because he could not determine whether the side of the primary thickening, where the secondary thickening appeared, was anterior or posterior; it appears probable, from what occurs in the development of other Arachnida, that this secondary thickening is the **primitive cumulus**, and that it appears at the hinder end of the primitive streak. Amongst the cells budded off from the primitive cumulus are probably the mother cells of the genital organs. It should be noted, however that Kautsch regards the so-called primitive cumulus as a thickening of no special significance, and expressly denies that it has anything to do with the genital organs—he regards it as separated from the *front* end of the primitive streak. The difficulty is that it has ceased to be recognizable before the first traces of segments have appeared in the embryo, before it has become possible to determine the long axis of the embryo with certainty.

After the secondary thickening has disappeared as a distinct mark, the primary thickening extends so as to form, when seen from the surface, an oval plate, which may be termed the **ventral plate**, on which the first rudiments of the future organs make their appearance. Transverse sections show that the proliferations of cells from the primary thickening form a longitudinal keel-like ridge projecting into the yolk. This keel-like ridge represents a solid invagination of endoderm. From the edge of the keel, cells are given off which wander through the yolk, increasing in size as they do so, and they eventually establish an epithelium on the outer surface of the yolk in the manner described below. These cells constitute the **permanent endoderm**. The rest of the ridge flattens out laterally and becomes divided into two parallel bands lying beneath the ectoderm; they constitute the **mesoderm** (Fig. 170).

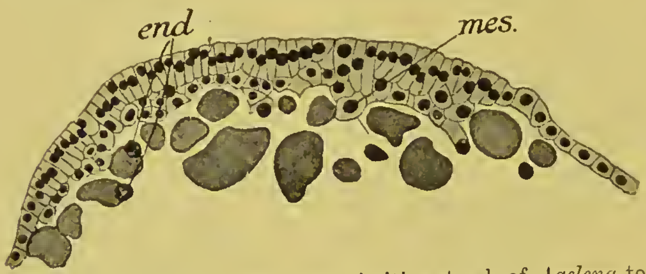


FIG. 171.—Section through the primitive streak of *Agelena* to illustrate the formation of the germ layers. (After Kishinouye.)

end, endoderm; *mes*, mesoderm.

Soon, as seen from the surface, the ventral plate becomes crossed by transverse grooves, which are the first indication of a division of the animal into metamerically arranged segments. In front there is an undivided **cephalic lobe**; behind, a **caudal lobe**; and the intervening plate is divided, in the earliest stage observed by Kishinouye, into five segments. Of these, the first corresponds to the segment from which the second pair of appendages, the pedipalpi, develop; the others correspond to the segments which bear the walking legs. The segment which gives rise to the first appendages or chelicerae develops later, being cut out from the cephalic lobe.

As development proceeds, further segments are cut out from the caudal lobe, till a total number of ten abdominal segments has appeared. The mesodermic bands have meanwhile undergone a corresponding segmentation into squarish blocks of cells, the somites, in each of which a coelomic cavity occurs. The tenth segment is not always distinct, nor does its somite always develop a cavity. Soon the rudiments of appendages appear as paired outgrowths from the segments, developing in order from before backwards, and a pouch from the corresponding coelomic sac extends into each of them.

The abdominal appendages grow slowly and only form rounded knobs; they do not grow nearly as quickly as the appendages on the other segments, and are absent on the seventh to tenth abdominal segments. The first abdominal segment gradually disappears; it possesses indications of a pair of appendages, but they never develop beyond the condition of slight elevations and soon disappear altogether. Very occasionally indications of a pair of appendages are found on the sixth segment. These also soon disappear.

The cephalic lobe, the origin of which has already been described, becomes divided into two semicircular lobes, in each of which a semicircular groove-like invagination is formed, which is almost certainly homologous to some extent with the "**cerebral grooves**" of *Peripatus* and *Astacus*. This invagination is the chief source of the cells of the brain; but there are also formed two invaginations at the posterior lateral border of the cephalic lobe, which become closed from the exterior and form little vesicles, and they also contribute to the building up of the brain. The rest of the nervous system appears as a series of paired thickenings of the ectoderm near the mid-ventral line, one pair appearing in each segment. The thickening in each segment is, however, only a more prominent portion of a continuous ridge-like thickening which extends through the whole length of the ventral plate and is continuous with the thickening which forms one side of the brain.

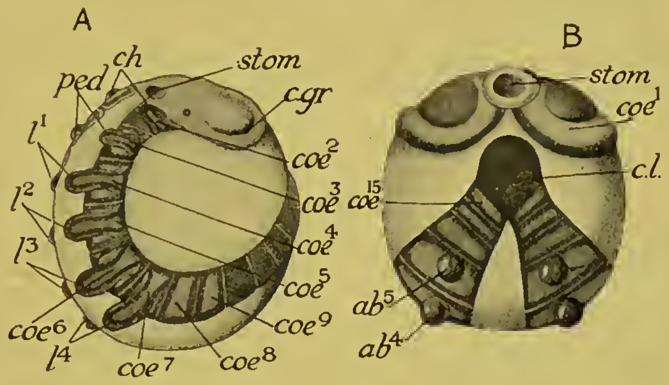


FIG. 172.—Two views of embryos of *Agelena* at the period of maximum extension of the ventral plate (*i.e.* before reversion has begun). The coelomic cavities are seen by their degree of transparence, and are represented by lighter shading. (After Kishinouye.)

A, lateral view of an embryo. B, dorsal view of a slightly older embryo than that represented in A. *$ab^{4\text{-}5}$*, the abdominal appendages belonging to fourth and fifth abdominal segments; *c.gr*, cerebral groove; *ch*, rudiment of chelicera; *c.l*, caudal lobe; *$coe^{1\text{-}15}$*, the coelomic sacs, from *coe^1*, the sac in the prae-oral lobe, to *coe^{15}*, the sac in the seventh abdominal segment; *$l^{1\text{-}4}$*, rudiments of the legs; *ped*, rudiment of the pedipalp; *stom*, stomodaeum.

The **stomodaeum** is now formed as an invagination just behind the cephalic lobes but in front of the chelicerae, and the **proctodaeum** appears considerably later, just in front of the tail-lobe, as a similar invagination.

When we compare the development so far recorded with that of *Peripatus* we must be struck with the fundamental resemblance. If, when the yolky endoderm cells of *Peripatus* have become invested by the ectoderm, the gut-cavity—which, it must be remembered, is formed by a process of secondary absorption—did not appear, then we should have a solid yolky endoderm, such as, to judge from the few observations that have been made on the subject, in point of fact appears to exist in *Peripatus novae-zelandiae.* (Sheldon, 1888, 1889.)

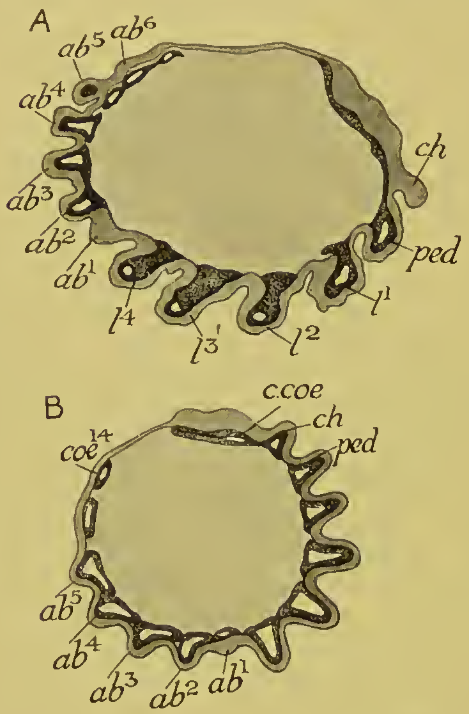


FIG. 173.—Two sagittal sections through embryos of *Agelena* of different ages, but previous to reversion. (After Kishinouye.)

A, younger stage. B, older stage. Letters as in previous figure. *c.coe*, cephalic coelom. In A no coelomic sacs have appeared in the cephalic lobe or in the cheliceral segment. In B the vestige of an appendage on the sixth abdominal segment present in A has disappeared.

The origin of the mesoderm as a pair of bands which become divided into somites, each containing a coelomic cavity, and the origin of the whole central nervous system as two ridges united in front of the mouth in the region of brain, and behind the position where the anus appears as an anal ganglion, is essentially the same both in the Spider and *Peripatus.* In the cephalic lobe of the Spider on each side there is a faint split in the mesoderm, which seems to be the rudiment of a coelomic cavity.

Whilst all this development has been going on, the ventral plate has grown in length till it almost encircles the ovum, the part of the circumference which represents the dorsal surface being of very small extent, and the caudal and cephalic lobes almost touching one another. Underneath this small dorsal surface there appear at the same time a number of cells gorged with yolk ("fat" cells). Previous observers had interpreted these to be wandering mesoderm cells and the forerunners of blood corpuscles, but Kishinouye interprets them as endoderm cells now for the first time reaching the dorsal surface of the yolk, though he admits that they develop into the first blood cells. Kautsch points out that at no time is it possible to discriminate sharply between mesoderm cells and yolk cells, since all intermediate stages between these two types occur.

During the period of development which succeeds the one which we have just described, a process termed "**reversion**" takes place. This consists in the development of the dorsal surface, which now begins to grow more quickly than the ventral one, forcing asunder the head and tail lobes, and at the same time forcing the mass of yolk which occupies the interior of the egg downwards between the two mesodermic bands. As a consequence, these bands, and the overlying ectodermic thickenings which constitute the rudiments of the right and left portions of the nerve cord, become widely separated from one another, and the very narrow band of ectoderm which occupies the mid-ventral line between corresponding thickenings of the right and left sides becomes enormously stretched.

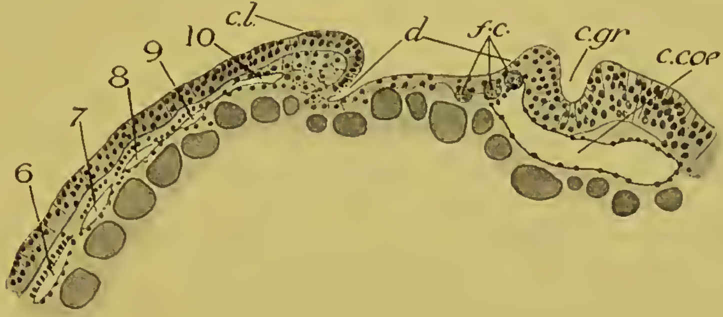


FIG. 174.—A portion of a sagittal section of *Agelena labyrinthica*, greatly enlarged. (After Kautsch.)

c.coe, coelomic sac in cephalic lobe; *c.gr*, cerebral groove; *c.l*, caudal lobe; *d*, dorsal surface; *f.c*, fat-cells; 6-10, the coelomic sacs in the abdominal segments 6-10.

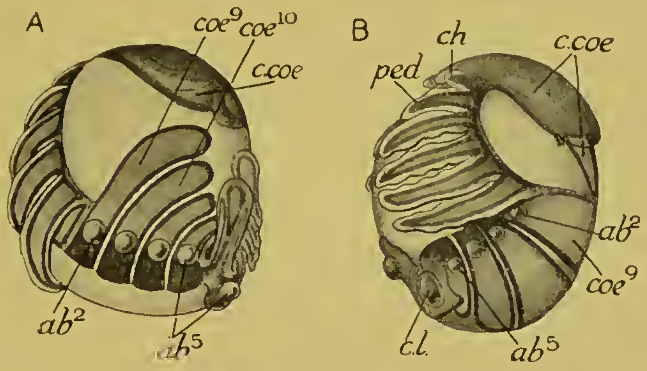


FIG. 175.—Two views of embryos of *Agelena* undergoing reversion. (After Kishinouye.)

A, an early stage of reversion. B, a later stage of reversion. ab^2, ab^5, the second and fifth rudimentary abdominal appendages. Other letters as before.

This process is one for which it is very difficult to find a mechanical explanation. Mere preponderant growth of the dorsal surface will not account for it. This by itself would only lead to the rucking up of the representative of the dorsal surface into an outstanding fold, just as the growth of the endodermic rudiment in the pathological blastulae of *Echinus*, produced by rearing the eggs in warm water, leads to the formation of a gut rudiment which projects from the surface of the blastula like the finger of a glove. Another suggested explanation of reversion is that it is conditioned by the pressure exerted on the egg by the tough chorion. The peculiarity about the process is that whilst the dorsal surface increases in extent it continues to form a part of the spherical surface of the egg; nay more, as it extends laterally it actually burrows under the mass of cells which form the origin of the caudal lobe, and causes this structure to appear as an outstanding projection. Kautsch points out, however, that if reversion is to be attributed to the increase in length of the dorsal surface of the embryo, the dorsal ectoderm cells should be under lateral pressure and should assume a columnar shape, whereas they are obviously passively stretched since they are exceedingly attenuated. He concludes that no mere mechanical explanation of reversion is possible.

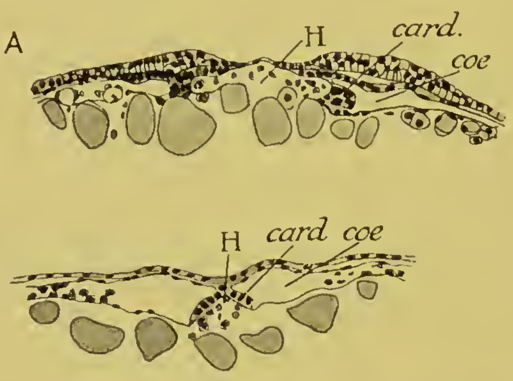


FIG. 176.—Transverse sections through two stages in the development of the heart of *Agelena labyrinthica*. (After Kautsch.)

card, thickened wall of apex of coelomic sac which forms wall of heart (the reference line is a little too short); H, heart-cavity.

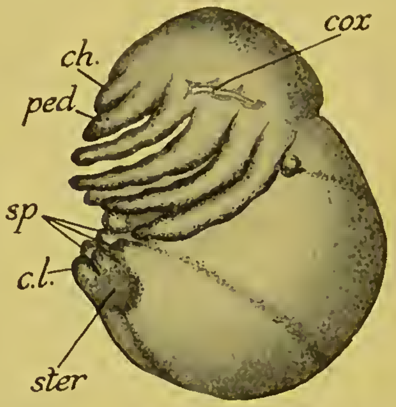


FIG. 177.—The embryo of *Agelena* when reversion is complete. (After Kishinouye.)

cox, coxal gland; *ster*, stercoral pocket; *sp*, spinnerets. Other letters as before.

During the progress of reversion many changes take place. The first abdominal segment, as well as the sixth, seventh, and eighth, have by this time disappeared. The coelomic sacs of the abdominal region extend dorsally till they meet one another in the mid-dorsal line. Below this point they do not meet, and so a space is enclosed between their dorsal apices which is the cavity of the **heart**. In this space are found many of the "fat" endoderm cells, alluded to above, which thus form the first blood corpuscles. The walls of the heart are formed by specially thickened regions of the walls of the coelomic sacs (*card*, Fig. 176). Since the coelomic sacs do not fuse with their successors, there occur gaps in the heart-wall between two segments.

These gaps are the origins of the **ostia** of the heart. The coelomic sacs belonging to the cephalic lobe also grow in a dorsal direction. They then become divided into dorsal and ventral portions. The latter lose their cavities and disappear, being probably transformed into connective tissue. The former meet in the mid-dorsal line and enclose between them a space which forms the **dorsal aorta**.

The coelomic cavities belonging to the six segments of the prosoma do not grow dorsally but extend further and further into the appendages, as these grow longer and become divided into joints. Soon the greater part of the cavities which are contained in the appendages disappear, their walls becoming converted into the extensor and flexor muscles which move each segment of the limb. The coelom disappears entirely in the segments belonging to the chelicerae and pedipalpi, but in the four segments which bear the ambulatory legs a small remnant persists in the base of each leg as a tiny vesicle. That portion of the coelom which is situated in the base of the first leg becomes connected with an ectodermic invagination which grows in to meet it. This invagination forms the duct of the **coxal gland**; the glandular portion of the organ is formed by the union on each side of all the coelomic sacs belonging to the four walking legs. This excretory organ may therefore be compared to one of the coelomiducts or so-called "nephridia" of *Peripatus*. The coelomic sacs which were situated in the first abdominal segment disappear; those which existed in the sixth, seventh, and eighth segments fuse into a single cavity on each side, which becomes pressed into the caudal lobe by the same force which presses the yolk mass downwards.

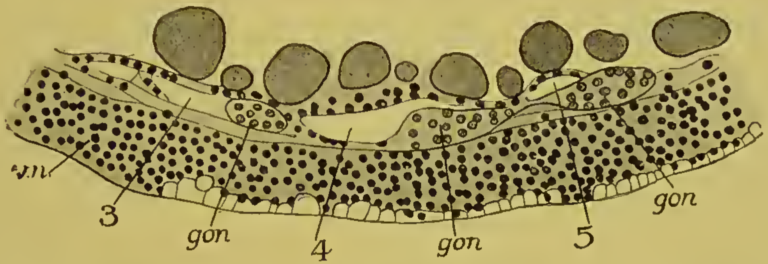


FIG. 178.—Longitudinal section through part of the abdomen of *Agelena labyrinthica*, in order to show the origin of the genital organs. (After Kautsch.)

3, 4, and 5, the coelomic sacs of abdominal segments 3, 4, and 5; *gon*, rudiment of genital organ; *v.n*, thickened ectoderm which gives rise to the ventral nerve cord.

The **genital organs** make their appearance as large pale cells in the walls of the coelomic sacs of the third, fourth, and fifth abdominal segments (Fig. 178). When the cavities of these sacs disappear these cells coalesce to form a compact mass on either side of the body which is the rudiment of the genital organ. The **genital duct** is formed by an outgrowth from the sac of the second abdominal segment and therefore, like the duct of the coxal gland, it is a coelomiduct.

The development of the genital organs of the spider bears a striking resemblance to their development in *Astacus*.

In the caudal lobe the first trace of the **mid-gut** now appears. An accumulation of yolk cells in the shape of a plate is formed. This plate becomes bent into a U-shape so as to enclose a cavity which is the rudiment of the **stercoral pocket** of the spider. Towards the main mass of yolk the stercoral pocket is closed by an accumulation of yolk cells. In this accumulation a cavity appears which develops into the hinder portion of the mid-gut, and which is, so to speak, a forward extension of the stercoral pocket. As it grows in length the stercoral pocket is pushed backwards. From the pocket itself two lateral outgrowths are given off which form the so-called **Malpighian tubes,** the excretory organs which the spider possesses in addition to the coxal gland (Figs. 179 and 180). The ectoderm of the ventral face of the caudal lobe is thickened so as to form the so-called **anal ganglia,** which arise at the posterior point of union of the two longitudinal rudiments of the nervous system. Just in front of this point the **proctodaeum** appears as an ectodermal invagination, and this invagination soon afterwards will open into the stercoral pocket.

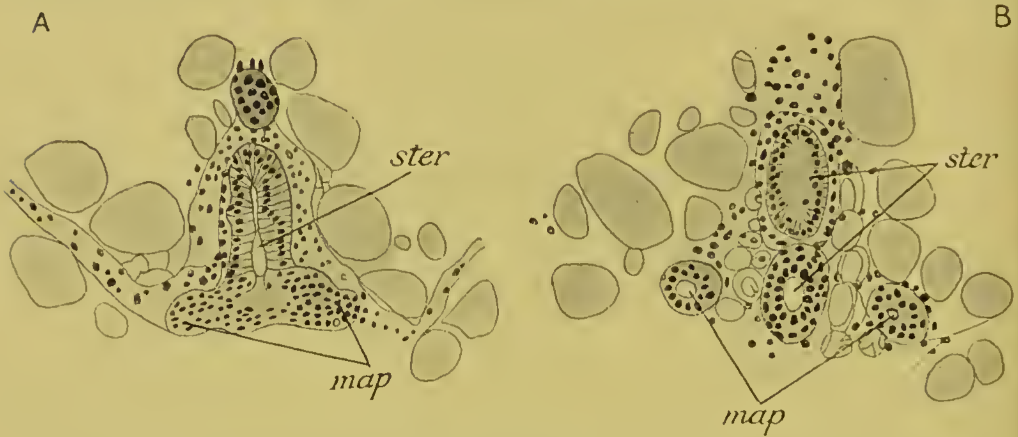


FIG. 179.—Two sections through the developing stercoral pocket and Malpighian tubes of *Agelena labyrinthica*. (After Kautsch.)

A, the posterior section. B, the anterior section. *map*, rudiment of Malpighian tube; *ster*, rudiment of stercoral pocket.

The persisting abdominal appendages now undergo further changes. On the posterior aspect of the first two pairs, near their bases, an ectodermic invagination is formed. In the case of the first pair this invagination forms the **lung sac.** This appendage develops on its upper and posterior face outstanding folds which are the rudiments of the first lung-lamellae. The other lamellae of the lung are formed by outgrowths from the thickened ventral wall of the lung sac—or to put it in another way, from the basal portion of the appendage. The credit of having given the first clear account of the development of the lung is due to Purcell (1909), whose account in all essential features has been confirmed by Kautsch (Fig. 181). In the case of the second pair of legs the invagination gives rise to the lateral **trachea.** The median trachea arises as a modification of the entopophysis or ectodermal tendon of the longitudinal abdominal muscle. The first two persisting abdominal segments are very broad, and hence the hinder segments are forced back to near the posterior end of the abdomen. The two hinder pairs of abdominal appendages become the **spinnerets** and in each a solid ectodermic invagination is formed at the apex of the limb, and gives rise to the **spinning glands.** The third pair of spinnerets are formed by the division of an inner lobe from the main mass of the last pair of abdominal appendages. They appear after birth (Fig. 182, C).

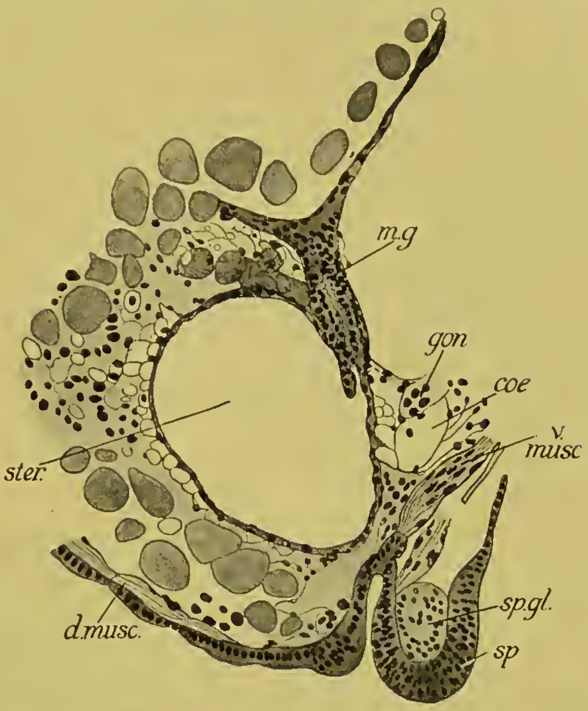


FIG. 180.—Sagittal section through the hinder part of the abdomen of *Agelena labyrinthica* to show the hinder part of the mid-gut developing in connection with the stercoral pocket. (After Kautsch.)

d.musc, dorsal longitudinal muscles; *m.g*, rudiment of hinder part of mid-gut; *sp*, spinneret; *sp.gl*, spinning gland; *v.musc*, ventral longitudinal muscles.

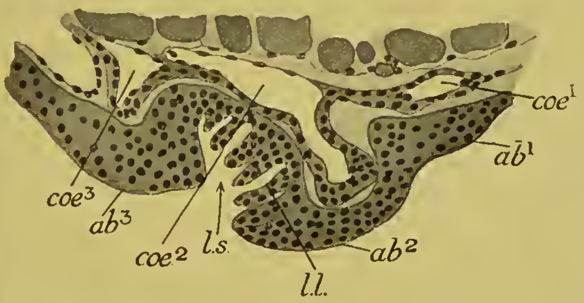


FIG. 181.—Longitudinal section through the abdominal appendages of an embryo of *Agelena labyrinthica*, to show the origin of the lung book. (After Kautsch.)

*ab*¹, *ab*², *ab*³, the first, second, and third abdominal appendages respectively; *coe*¹, *coe*², *coe*³, the coelomic sacs belonging to the first three abdominal segments; *l.l*, lung lamellae; *l.s*, lung sac.

The cephalic lobes have now fused in the middle line, and their semicircular grooves have also fused at one point. These grooves now begin to be closed in from the exterior, and the last portions to remain open are the most posterior parts of their inner limbs. Just above the spots where these grooves finally close, a pair of ectodermic invaginations mark the site of the **central eyes.** The **lateral eyes** are formed as depressed circular areas in the ectoderm, one on each side of the head, *i.e.* at the edges of the ventral plate. The lateral vesicles are now completely cut off from the exterior, and Kishinouye compares them to the eyes of *Peripatus.*

The chelicerae have been shifted forward so that their bases nearly meet in the mid-ventral line in front of the mouth, and their ganglia form the commissure which connects the brain with the large **suboesophageal ganglion.** The latter is formed from the ganglia of the pedipalps and the four ambulatory limbs; these ganglia are in close contact with one another, but at this stage have not yet fused.

The above concludes the account of the changes which take place during the process of reversion. When this process is complete the undigested yolk forms a huge semicircular protrusion on the ventral surface, and the mesodermic bands are situated somewhere about the equator of the egg, which still retains its spherical form.

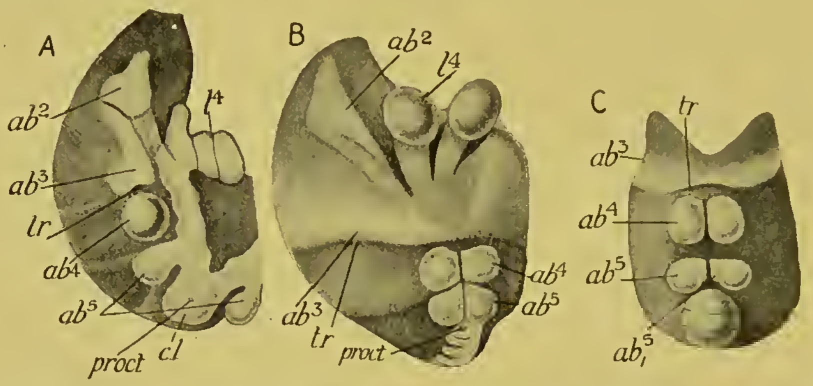


FIG. 182.—Surface views of the cut-off abdomen of three embryos of *Agelena labyrinthica* of different ages in order to show the modifications undergone by the abdominal appendages. (After Kautsch.)

ab^2-ab^5, the abdominal appendages; ab^5 (on the right side of Fig. C), the inner part of the last abdominal appendage, which gives rise to the third spinneret; *c.l*, caudal lobe; l^4, the last leg; *proct*, proctodaeal invagination: *tr*, invagination to form the trachea.

In the succeeding period of development, which lasts until birth, the yolk becomes absorbed, and as it disappears the ventral ectoderm contracts in width, and the two halves of the nerve cord and the two mesodermic bands approach one another once more. At the same time the ventral ectoderm grows in length and forms a deep inwardly directed fold which is the beginning of the constriction separating abdomen and prosoma from one another. During this period the various organs complete their development.

We may commence by the consideration of the central nervous system and eyes. The grooves in the brain become now completely closed off from the exterior and form crescentic tubes. The inner portions of these crescentic tubes completely fuse with one another so as to form the stem of a T, the transverse arms of which are formed by the outer portions of the grooves. The walls of the tubes form the substance of the brain, and this organ shows a distinct division into three segments. Of these the most anterior is formed from the cross beam of the T, the other two form the main stem.

The central eyes arise as pits just behind the posterior ends of the brain grooves, they belong, therefore, to the hindermost segment, while the lateral eyes are situated even still further behind this point. Patten's attempt to show that a pair of eyes belong to each segment is, therefore, unjustified.

The terms anterior and posterior must be used with reference to the mutual positions of the segments as they lie in the anterior portion of the ventral plate. By the growth in length of the ventral ectoderm the segments become pushed up round the anterior end of the animal on to its dorsal surface, so that what was anterior on the ventral surface becomes posterior on the dorsal. Thus the median eyes attain a position behind the lateral eyes, although the latter are morphologically posterior to them.

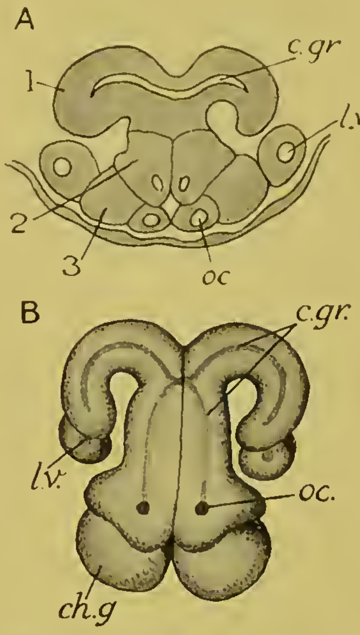


FIG. 183.—The condition of the brain in the embryo of *Agelena* after reversion has taken place. (After Kishinouye.)

A, frontal section of the brain and adjacent structures. B, diagram of the brain; 1, 2, 3, the three segments of the brain; *c.gr*, cephalic groove (now closed to form a tube); *ch.g*, cheliceral ganglion; *l.v*, lateral vesicle; *o.c*, rudiment of central eye.

The lateral eyes on each side originate as a simple ring-like pit of ectoderm which becomes divided by the continuance of the process of invagination into several deeper secondary pits, and each of these becomes closed off from the exterior by the constriction of its opening. The floors of these secondary pits are directly converted into retinulae, their component cells becoming visual cells. Between the upper ends of the visual cells, rhabdomes or visual rods are formed, whilst their lower ends are directly converted into nerve fibres which become connected with the brain. The roofs of these pits are formed by overfolding of the ectoderm, and constitute the vitelligenous cells which secrete the crystalline bodies between them, whilst over all the general cuticle is continued. The cuticle, which is of course secreted by the ectoderm, is thickened where it covers the eye, forming there a lens.

The central eyes have a different fate. When the vesicles out of which they develop become closed, the last trace of the opening of each vesicle is situated posteriorly, and each vesicle, therefore, consists of an upper and an under wall with a slit-like cavity between them.

From the upper, not the lower of these two layers, the visual cells are developed, and from the ectoderm which covers the whole sac, the vitelligenous cells are formed. It follows that what were originally the outer ends of the visual cells are turned away from the light, whilst their basal ends, from which the nerve fibres spring, are turned inwards. The lower end of the sac forms the **post-retinal** layer (*p.r*, Fig. 184).

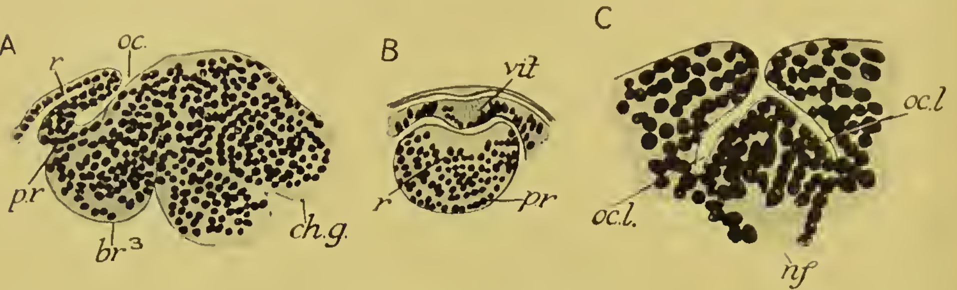


FIG. 184.—Sections through the developing eyes of *Agelena*. (After Kishinouye.)

A, section through early stage of development of the median eye. B, section through later stage of development of median eye. C, section through invagination from which the lateral eyes develop. *br*³, third lobe of the brain ; *ch.g*, cheliceral ganglion ; *n.f*, nerve fibres ; *oc*, rudiment of median eye ; *oc.l*, rudiment of one of the lateral eyes ; *p.r*, post-retinal layer of cells ; *r*, layer of visual cells ; *vit*, vitelligenous layer.

The nervous system before and during reversion is in the form of a thickening of the ectoderm ; it now becomes detached from the ectoderm and the ganglia of the ventral chain soon fuse into one suboesophageal mass (Fig. 185, B). The characteristic **poison gland** is developed as an ectodermal ingrowth in the base of the chelicera.

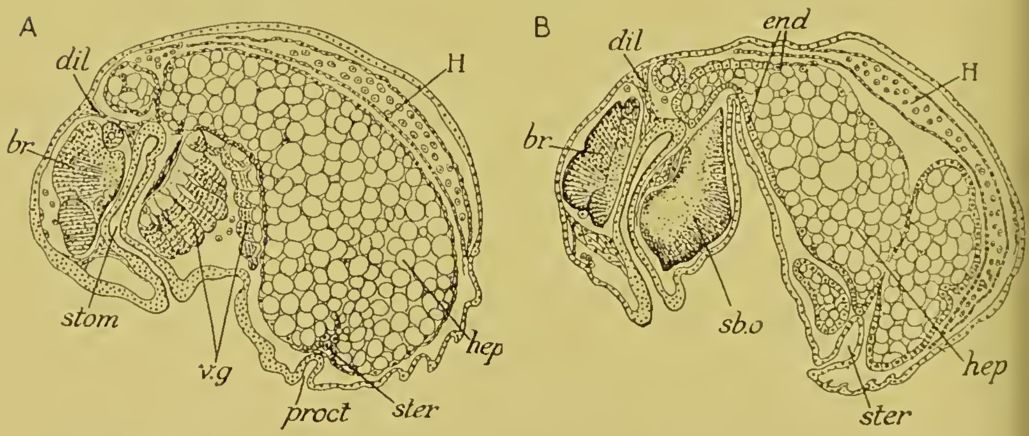


FIG. 185.—Two sagittal sections through embryos of the spider *Theridion maculatum*, in two succeeding stages of development. (After Morin, from Korschelt and Heider.)

A, ventral nerve cord, showing ganglia. B, ventral ganglia fused to form a suboesophageal mass. *br*, brain ; *dil*, dilator muscle of "stomach" (*i.e.* stomodaeum) : *end*, endodermic epithelium beginning to cover the mass of yolk ; *H*, heart ; *hep*, liver ; *proct*, proctodaeum ; *sb.o*, suboesophageal ganglion ; *ster*, stercoral pocket ; *stom*, stomodaeum or stomach ; *v.g*, ganglia of the ventral nerve cord.

The stomodaeum develops rapidly, sloping upwards and backwards.

Its innermost portion enlarges to form the so-called **stomach**, whilst its outer portion forms the **pharynx** and has its cuticular lining produced into parallel ridges. The **oesophagus** is the narrow portion connecting the pharynx and stomach. At the hinder end of the stomach an accumulation of endoderm cells is found abutting on the yolk, which now becomes indented by the ingrowth of four pairs of mesodermic septa, and the yolk lobes thus outlined eventually form the lobes of the **liver.** These septa seem to be the outgrowths from the abdominal coelomic sacs, whose cavities have disappeared.

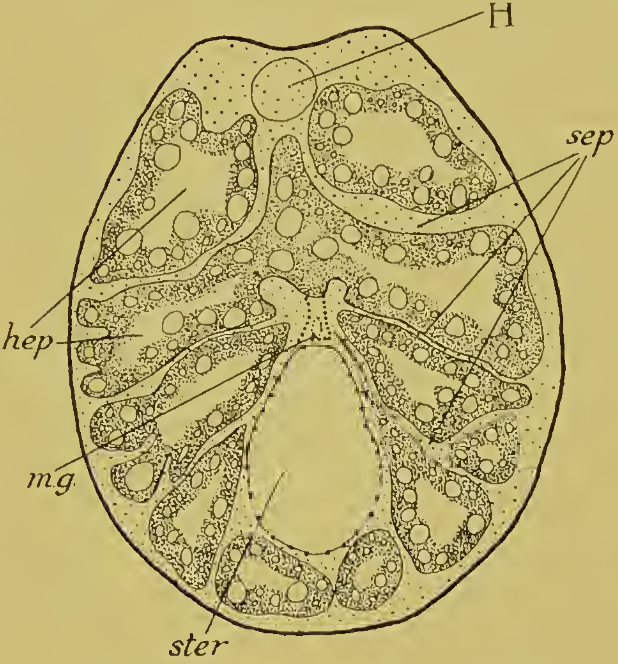


Fig. 186.—Horizontal section through abdomen of an advanced embryo of *Agelena labyrinthica* to show the division of the yolk into lobes by septa. (After Kautsch.)

H, heart; *m.g*, hinder part of mid-gut; *sep*, mesodermic septa dividing the yolk; *ster*, stercoral pocket.

Kautsch maintains that the epithelium covering the lobes of the liver is derived from the mesodermic cells of these septa. This is excessively unlikely, and Kishinouye's statement that it is derived from the anterior accumulation of "yolk cells" situated where the endoderm abuts on the yolk, and which, according to him, gradually spread throughout it and rise to the surface, is infinitely more likely. In *Astacus* we have already learnt that the liver lobes arise as a result of the indentation of the yolky endodermic sac by mesodermic septa, but in that animal there is no question that the epithelium of the liver arises from the endodermic nuclei and their surrounding cytoplasm, and this is also probably the case in the spider. The appearance of the lumen of the mid-gut first in the region of the stercoral pocket is paralleled in *Astacus* by the first appearance of a definite "endodermal plate" in the hinder region of the mid-gut.

The development of the embryo is now complete and it is hatched.

OTHER ARACHNIDA

What is known of the development of other Arachnida demonstrates that it is in remarkable agreement with that of the spider, in all essentials, but it is only in the case of *Limulus* and the scorpion that even an approximately complete account of the life-history has been elucidated.

If we turn to Kishinouye's account of the development of *Limulus* (1893) we find that for the earliest period of development only an incomplete series of stages was at his disposal, and that, if his conclusions are correct, Limulus differs from the spider inasmuch as the nuclei which represent the **endoderm** are budded into the yolk before the keel of the primitive thickening is formed. Kingsley (1892–1893) describes the cells forming the blastoderm as dividing tangentially into a small clear moiety which remains at the surface, and a large inner half, full of yolk grains, which is endodermal and wanders off into the yolk.

The primitive thickening, when it appears, is said to give rise only to **mesoderm**. A similar statement, as we shall see later, has been made for insect development, but it seems clearly to be erroneous for insects, and may prove to be so also for *Limulus*. We must bear in mind that the epithelium of the gut of *Limulus* is formed very late, long after the larva is hatched, and that it appears first of all in the region of the abdomen. Further, no coelomic sacs at all appear in the bases of the appendages of the prosoma, except in the fifth and sixth, and these form the **coxal gland.** It follows that the coxal glands of *Limulus* and the spider are not strictly homologous with one another, but that both are remnants of a once complete series of metamerically arranged excretory organs.

As there is no anterior aorta in *Limulus* we find that the coelomic sacs of the cephalic lobes do not meet each other, but that a vascular circumoesophageal collar is formed by the shrinkage of these sacs from the sides of the oesophagus. The **heart** is formed in exactly the same way as in the spider. According to Kingsley, after giving rise to the heart-wall the coelomic sacs fuse longitudinally with their predecessors and successors, and so two persistent tubes are formed which, he thinks, give rise to the **genital organs**, as they do in *Peripatus* (see p. 175).

The dorsal and ventral surfaces develop in even proportion with one another, so that there is no process of reversion. The last appendage of the prosoma, in the adult, has an outer branch called the **epipodite** or **flabellum**, which is probably the remnant of an **exopodite**, such as forms the major part of the limb in the case of the appendages of the abdomen. *In the embryo all the appendages of the prosoma except the first, develop the beginnings of this exopodite, but it only persists in the sixth and last.* (Fig. 187, *flab*$^{1-5}$.)

The **central eyes** of *Limulus* are inverted like the posterior central eyes of the spider, and originate from a corresponding part of the ectoderm; in front of the brain rudiment on the ventral plate, measuring along the ventral surface, behind if we measure on the dorsal surface. The **lateral eyes** of *Limulus* originate also as a single undivided pit on each side, as such pits begin in the spider; but though they develop many ommatidia, formed by the grouping of some of their cells to form retinulae, they do not become subdivided into smaller eyes (Fig. 187, *oc.l.*), each remains as an undivided compound eye throughout life, and consists throughout of a single layer of cells; there is no vitelligenous layer. The lateral eyes are developed from the sides of the cephalic lobe, and as growth proceeds the cephalic lobe extends backwards along the sides of the prosoma, so that the eye appears as if it belonged to the fourth segment.

The appendages of the abdomen appear as plates, each with an inner lobe which we may regard as the endopodite; the first pair of appendages becomes, however, progressively reduced in size, and the segment to which they belong ceases to be distinguishable. Remnants of these appendages remain as "**chilaria**" forming an underlip. The second appendage joins its fellow to form the **genital operculum**, the rest form **gill books**.

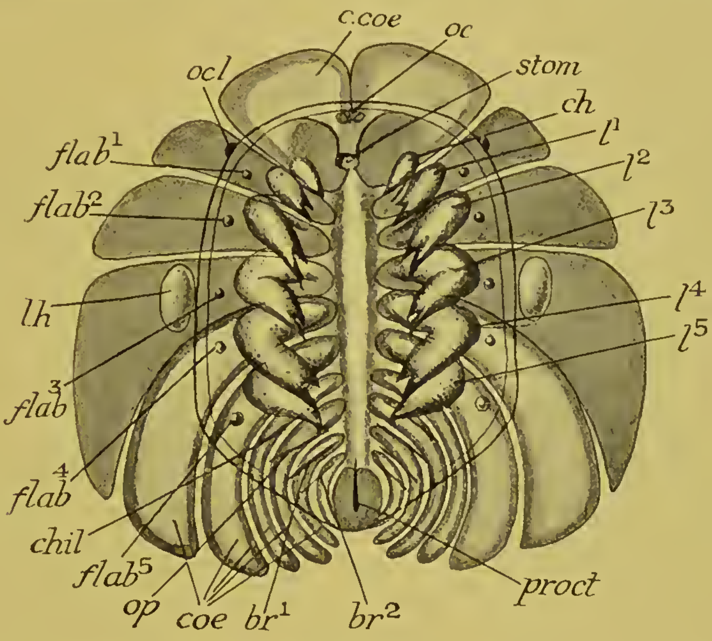


FIG. 187.—Ventral view of an embryo of *Limulus longispina*, 21 days old. (After Kishinouye.)

br^1, appendage bearing first gill book; *br*^{2}, appendage bearing second gill book; *ch*, chelicera; *chil*, chilaria; *c.coe*, cephalic coelom; *coe*, coelomic sacs; *flab*$^{1-5}$, the rudiments of five pairs of flabella, *i.e.* of exopodites; *l*^{1}-*l*^{5}, the rudiments of the five pairs of walking legs; *l.h*, lateral hump; *oc*, median eye; *oc.l*, lateral eye; *op*, genital operculum; *proct*, proctodaeum; *stom*, stomodaeum.

The embryo is hatched out as the so-called "**Trilobite larva**" which gradually changes into the adult. In this larva the appendages have all attained their adult form, but the abdomen is still distinctly divided into segments, and each segment, seen from above, shows a median **tergum**, and, on each side, a horizontally extended **pleuron**, separated from it by a groove like the body segment of a Trilobite seen from above. In the region of the prosoma we have similarly a median "**glabellum,**" consisting of the fused terga of the prosomatic region, and on each side of it a "**fixed cheek,**" consisting of the fused pleura of this region. Outside the fixed cheek there is a marked suture or joint line, the "**facial suture,**" which runs round in a semicircle parallel with the edge of the carapace. The cephalic lobe and its lateral extensions, which we may term "**free cheeks,**" lie beyond, and in these latter we find the compound lateral eyes (Fig. 188).

Point for point this structure is repeated in the cephalic shield of a Trilobite, and so far the agreement between the Trilobite and the larva is complete. But since the appendages of the Trilobite have been made known to us by Beecher (1893) it is at once seen what a wide divergence there is between them and those of *Limulus*. The Trilobite has unforked filiform antennae, and all the other appendages are similar to one another. All possess a jointed endopodite with a gnathobase, all have a rod-like exopodite which carries a "book" of long narrow gills, essentially similar to those of *Limulus*, but the contrast between the appendages of the prosoma and those of the abdomen, which is so marked a feature in *Limulus* as in all true Arachnida, is utterly absent in the Trilobite; moreover, *Limulus* shows no trace of the antennae of the Trilobite.

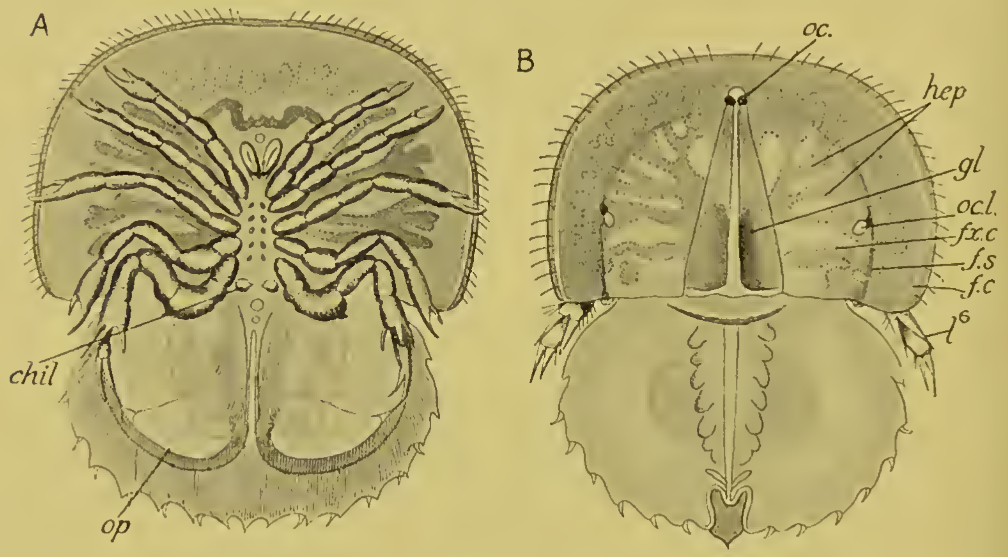


FIG. 188.—The Trilobite larva of *Limulus*. (After Kingsley.)

A, ventral view. B, dorsal view. *f.c*, free cheek ; *f.s*, facial suture ; *fx.c*, fixed cheek ; *gl*, glabellum ; *hep*, lobes of liver seen through ; l^6, the last walking leg ; *op*, genital operculum.

In a word the so-called Trilobite larva represents not a Trilobite but an Arachnid, not very unlike the adult but with segments which are quite free from one another, and without the long tail. Such Arachnida are known to have existed in the Silurian epoch (*Hemiaspis*), but the stock from which Arachnida and Trilobita diverged must be still further back in the remote past.

As regards the Scorpion, the embryology of which has been worked at by many observers, the latest of whom is Brauer (1894, 1895), we find again serious modifications in the early development as compared with that of the spider. The eggs of the scorpion are not laid, but are retained within the body of the mother until development has so far advanced that the young, when born, have most of the features of the adult. The nucleus of the ripe egg is situated not in the interior of the egg but at its surface, and the daughter nuclei, which result from its division, form at first a single-layered blastoderm extending over only a portion of the surface of the egg. The egg is therefore telolecithal and its segmentation is meroblastic, but this type of telolecithal egg, as we can see by comparing it with the egg of the spider, must have been secondarily derived from the centrolecithal type.

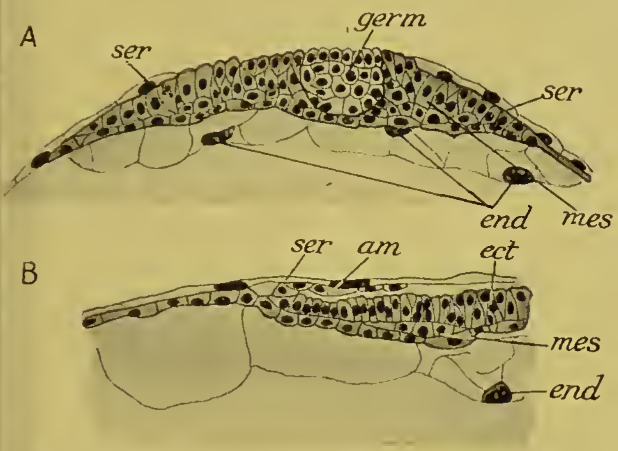


FIG. 189.—Two transverse sections through the "germinal disc," or developing area of the egg of the Scorpion, *Euscorpius carpathicus*, in two stages. (After Brauer.)

A, Stage of the formation of the serosa. B, stage of the formation of the amnion. *am*, beginning amnion; *ect*, ectoderm; *end*, endodermal nuclei; *mes*, mesoderm; *germ*, primitive germ cells, probably corresponding to the "primitive cumulus" of the spider's egg.

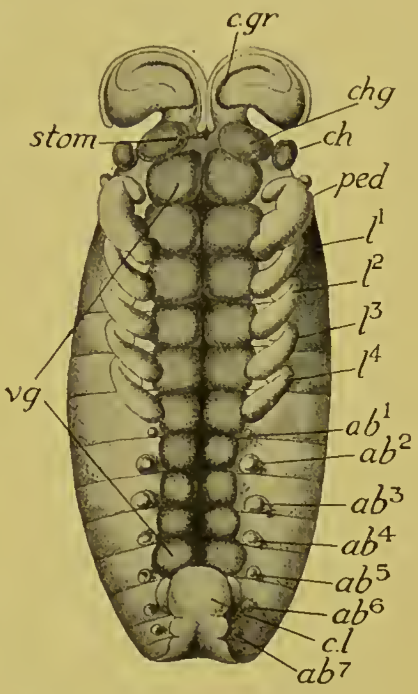


FIG. 190.—Ventral view of embryo of the Scorpion *Euscorpius carpathicus* showing segments and appendages. (After Brauer.)

ab$^{1-7}$, the rudiments of the abdominal appendages; *ch*, rudiment of chelicera; *ch.g*, rudiment of the cheliceran ganglion; *c.gr*, cephalic groove; *c.l*, caudal lobe; *l*$^{1-4}$, the rudiments of the walking legs; *ped*, rudiment of pedipalp; *stom*, opening of stomodaeum; *v.g*, ganglia of the ventral nerve cord.

At a later period cells are budded off from the blastoderm which wander into the yolk. These cells, according to Brauer, eventually form the **endodermal** epithelium, at a much later period in the development. At the same time the edges of the blastoderm give rise to a sheet of cells which grows backwards over it and forms a protective cover for it, called the **amnion**. A little later, a second outer covering of the same kind is formed which is called the **serosa** (Fig. 189).

A keel is formed by a thickening of the blastoderm as in the spider; from this keel a layer of cells grows outward on each side beneath the ectoderm, as in the spider and in *Limulus*, and forms the **mesoderm.** Behind this primary thickening there is a second thickening formed, as Kishinouye has also described for the spider. Brauer, however, asserts that in the scorpion this secondary thickening, or **primitive cumulus** as it is called, gives rise to a group of cells which remains unchanged for a great period of development and then gives rise to the **genital cells.** There is strong presumption that this will eventually be found to be true in the case of the spider also.

The general history of the later development of the scorpion is very similar to that of the spider in its main outlines, but the following points are to be noted. The egg is cylindrical and the ventral plate only occupies one side, consequently there is no need for reversion. But since the blastoderm only covers a portion of the surface and is reflected at its edges to form protective membranes, the covering of the dorsal surface of the egg with skin is effected by the lateral growth of the ventral plate, and the pushing of its right and left edges (that is to say, the lines of origin of serosa and amnion) farther and farther up towards the dorsal surface, till they meet on the mid-dorsal line, results in the protective membranes being cut off from the egg.

Brauer could only distinguish two segments in the brain, each marked by a transverse commissure. The lateral eyes remain as open pits of epithelium, and they have no vitelligenous layer. The so-called coelomic cavity of the head or cephalic lobe is, according to him, merely an extension of the coelomic sac belonging to the segment of the chelicera, as may also be the case with both spider and *Limulus*, in spite of Kishinouye's statement; or perhaps Brauer is mistaken and has confused subsequent fusion with common origin.

The **Malpighian tubes** are outgrowths of the posterior end of the mid-gut. Rudiments of **excretory organs** or so-called "nephridia" are formed as outgrowths from the coelomic sacs in all the segments of the prosoma from the second to the sixth, but only that one in the fifth segment comes to full development and it forms the **coxal gland.** The coelomic sacs of the abdomen press on the cells which form the genital rudiment, and eventually these cells pass into the interior of the first coelomic sac of this region of the body. The **genital ducts** arise in exactly the same way as the "nephridia," with which they are no doubt serially homologous.

The abdominal appendages form at first freely projecting plates, but the first abdominal segment has only vestiges of appendages which soon disappear and the whole segment then becomes indistinguishable; the appendages of the second segment form the **genital operculum,** those of the third pair form the "**combs**" or **pectines.** The skin behind the fourth, fifth, sixth, and seventh pairs of appendages becomes tucked in so as to form the **lung sacs,** and folds on the anterior surfaces of these sacs form the **lung books.**

All the nerve ganglia of the prosoma and the first pair of the abdominal ganglion fuse to form the **suboesophageal ganglion.**

The **heart** is formed, just as in the spider, by the meeting of the dorsal ends of the coelomic sacs of opposite sides. A semicircular plate of cells is detached from the apex of each of the two coelomic sacs, and the two grow together to enclose a blood space which is the heart. Brauer has described in detail how the **pericardium** is formed. The two semicircular plates become completely detached from the coelomic sacs of which they once formed part, these latter shrink away from the heart and meet beneath it, and the space which is left between heart and conjoined coelomic sacs is the pericardium. The coelomic sacs then lose their cavities by a process which consists in the development of fibrils crossing their lumen, and the swelling up and rounding of the cells forming their walls, and the comparatively solid plate of connective tissue which results forms the **pericardial septum** or floor of the pericardium. A similar process then occurs in the remaining parts of the coelomic sacs, and the only portions of their cavities which persist are found in the coxal glands, and presumably in the genital organs, though the origin of these latter was not traced.

What little is known of the course of development in other orders of Arachnida fits in well with what is known of the scorpion, spider, and *Limulus.* The formation of sheets of cells acting as protective coverings to the developing embryo is not known to occur except in the scorpion. In all cases, however, an indication of the primitive cumulus at the hinder end of the primitive streak can be made out, and in the Pedipalpi, according to Schimkewitsch, the formation of mesoderm takes place from the cumulus alone. Thus the cumulus cannot merely represent the genital rudiment but must represent the primitive streak of *Peripatus, i.e.* the obliterated section of the blastopore which occurs behind the anus in that animal. For a similar reason the primitive streak of Arachnida must represent the open portion of the blastopore of *Peripatus.*

In the Acarina or Mites the young are hatched in an imperfect form in which only three pairs of walking legs are developed. After living in this state for some time they moult a thick cuticle, which, however, remains surrounding them like a second egg-shell, called the **deutovum,** inside which further development takes place and the missing fourth pair of legs make their appearance. This reduction of a series of homologous organs to a smaller number, in accordance with the minute size of the embryo when hatched, is entirely in line with what we have learnt of larval modification amongst Crustacea.

The Pentastomida, which, even when adult, are parasitic in the nasal cavities of the dog, are supposed to be the extreme limit of degeneration in Acarina. The larva, which encysts itself in the connective tissue of rabbits, sheep, etc., exhibits the rudiments of two pairs of appendages.

PANTOPODA

The Pantopoda, or sea-spiders, are treated by most zoologists as a quite independent group of Arthropoda. Huxley, however, regarded them as aberrant Arachnida. They agree with Arachnida in possessing no proper jaws and in having four pairs of walking legs, but differ from all Arachnida in the absence of the division of the body into a prosoma with leg-like appendages in front, and an abdomen with plate-like appendages behind, behind one of which the genital ducts open.

In the Pantopoda the so-called abdomen is an unsegmented stump devoid of appendages, and the genital ducts, of which there may be several pairs, open at the bases of the long legs. Further, the three front segments bear pairs of legs reduced in size and not used for walking; behind these come four pairs of walking legs, but since the fourth of these is the seventh appendage it cannot correspond to the last walking leg of Arachnida, though it may correspond to the suppressed segment of *Limulus* and the scorpion.

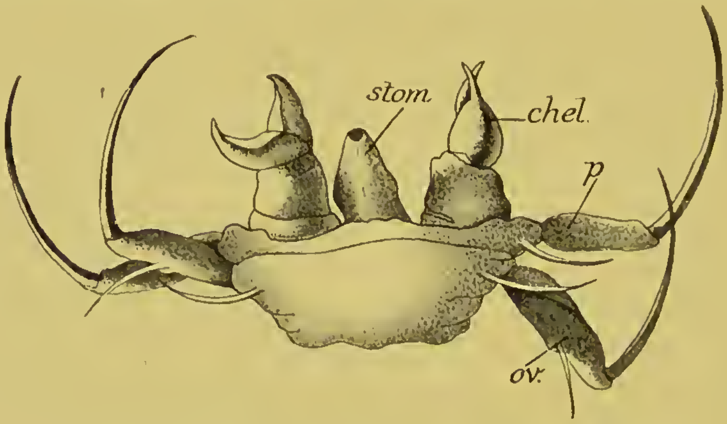


FIG. 191.—Larva of *Ascorhynchus minutus*. (After Hoek.)
chel, chelophore ; *ov*, ovigerous leg ; *p*, palpus ; *stom*, stomodaeal proboscis.

The most plausible suggestion as to the origin of the Pantopoda is that they represent a divergent branch of primitive Arachnida at the time that these were separating from the common stock of all Arthropoda.

The development of these interesting forms is very imperfectly known, but what little is known only whets the desire to know more. Thus in *Pallene*, according to Morgan (1891), the egg exhibits a complete segmentation, and a **blastula** results, surrounded by a few large columnar blastomeres and provided with a small blastocoele. Later, however, just as in *Branchipus* and *Astacus*, the inner ends of these blastomeres coalesce to form an unsegmented mass of yolk. It appears that the endoderm is formed by budding off cells into the yolk, and it seems likely (though Morgan denies it) that this budding takes place in connection with a small invagination which occurs at one pole. From the lips of this invagination at any rate the **mesoderm** is developed.

It would appear that the early development of *Pallene* bears some considerable resemblance to that of *Palaemon* (see p. 192). *Pallene* emerges from the egg when it has almost attained the adult condition, but most Pantopoda emerge as larvae with three pairs of legs and pursue a semiparasitic life inside Hydroid polyps, gradually attaining the adult condition after a series of moults, at each of which a new pair of legs is developed.

TARDIGRADA

A word or two may here be interposed about the development of the Tardigrada, though it is exceedingly doubtful whether these minute, degenerate Arthropoda are really related more closely to Arachnida than to the other groups. They possess no jaws or antennae and only four pairs of stumpy unbranched appendages. The development of one species, *Macrobiotus macronyx*, has been worked out by Erlanger (1895). He asserts that the minute egg undergoes total segmentation, and that a hollow **blastula** is formed from which a **gastrula** arises by invagination. From the archenteron four pairs of **coelomic sacs** arise as hollow outgrowths, and there is also a median posterior sac arising in the same way, from which **genital organs** and **Malpighian tubules** arise. It is possible, however, that Erlanger's account of the development of the coelom is incorrect, as it was founded on whole mounts and not upon sections.

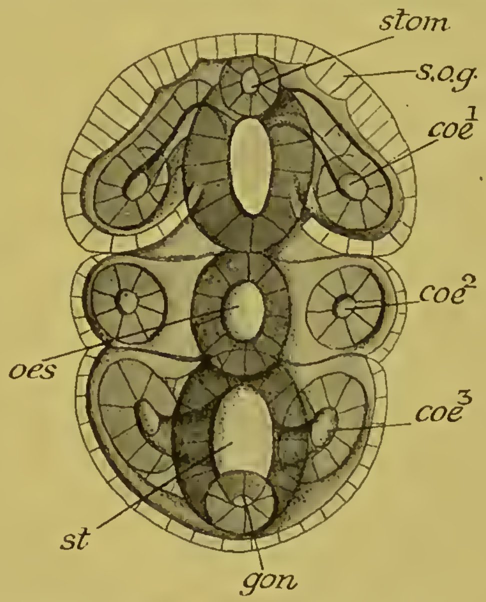


FIG. 192.—Dorsal view (optical frontal section) of embryo of *Macrobiotus macronyx*. (After Erlanger.)

coel-3, coelomic sacs, of which the first and the last can be seen to open into the gut; *gon*, terminal coelomic sac, which is the rudiment of the gonad; *oes*, oesophagus; *s.o.g*, ectodermic thickening, rudiment of supra-oesophageal ganglion; *st*, stomach; *stom*, rudiment of the stomodaeum.

ANCESTRAL HISTORY OF THE ARACHNIDA

When we survey the development of Arachnida so far as it is known, we are struck by a fundamental agreement in type in animals so diverse as *Limulus*, a scorpion, and a spider. In all of them the yolk is so abundant that no trace of a complete segmentation of the egg into blastomeres persists; segmentation is represented by multiplication of nuclei and their arrangement at the surface of the egg. The first differentiation of layers takes place in connection with a ventral thickening of the blastoderm, which may be regarded as representing the blastopore of *Peripatus*. The mesoderm soon becomes split into two bands, right and left, and in each of these a series of coelomic pockets makes their appearance.

It is difficult to decide whether Kishinouye is right in asserting that the first pair of these pockets is found in the cephalic lobes, and is distinct from the pair which appears later in the segment bearing the chelicerae, or whether Brauer is right in asserting that the coelom of the cephalic lobe is a mere forward production of the coelom of the cheliceral segment.

If Kishinouye is right it is quite probable that the coelomic sacs in the cephalic lobe represent a lost anterior segment in these animals, a segment which is in front of the segment corresponding to the first antennae of *Peripatus*, centipedes, and insects. As we shall see later, this lost segment is distinctly represented and bears vestigial appendages in the embryo of the centipede. We may provisionally accept Kishinouye's view, remarking merely that it seems clear that most interesting results would be obtained by a revision of his work with the aid of modern methods, when many of these vexed questions might be solved.

We form, therefore, the following picture to ourselves of the manner in which Arachnida were developed. They arose from ancestors in which all the segments except the first bore bifurcated appendages, with plate-like exopodites and more or less leg-like endopodites. The first pair of appendages, however, had been modified into antennae, or tactile organs, and were subsequently lost. The succeeding appendages had their endopodites modified into walking and grasping organs and had lost the exopodites, whilst the hindermost appendages retained their plate-like form and assumed respiratory functions. Such ancestors must have closely resembled Trilobites, but their divergence from that group consisted in this, that in Trilobites, and to a still greater extent in Crustacea, the appendages immediately following the antenna tended to become **gnathites** (or jaws), by the diminution in size of their distal members and the development on their proximal members of cutting blades; and this process went only a very small way in Arachnida, in which the corresponding appendages functioned as the main organs of locomotion.

The plate-like form of limb is no doubt the original form in Arachnida as it is in Crustacea; and the process of transforming these plates into rounded legs, to which the differentiation of the front part of the body of an Arachnid is due, began in front and travelled backwards. Pantopoda probably represent a group in which it went farther back than in Arachnida, and in which the hindermost appendages were lost.

INSECTA

Classification adopted (only those orders specially alluded to in the text are mentioned)—

Aptera	**Thysanura**
	Collembola
Hemimetabola	**Orthoptera**
	Paraneuroptera (Odonata)
	Ephemeroptera
	Hemiptera
Holometabola	**Coleoptera**
	Lepidoptera
	Hymenoptera
	Diptera

The Insecta, even if we confine ourselves to Insecta Hexapoda and exclude Myriapoda, are an enormous group, including nearly three hundred thousand named species. A large amount of work has been done on their development since the earliest days of scientific embryology, and a full discussion of this would lead us entirely beyond the limits assigned to this work. Fortunately, in comparatively recent times the embryology of two species has been worked out in a thoroughly satisfactory manner, viz. that of *Doryphora* (*Leptinotarsa*) *decemlineata*, the so-called potato bug, or Colorado beetle, by Wheeler (1889), and that of *Donacia crassipes*, by Hirschler (1909), a beetle belonging to a closely allied family and abundant throughout Europe. The results of these two investigators are in agreement in all important points, but as Hirschler's work is the most recent, we shall select *Donacia* and not *Doryphora* as type.

DONACIA

The eggs of both forms are laid in batches enclosed in a cocoon and attached to the under surface of leaves, those of *Doryphora* to the leaves of the potato plant and allied forms, those of *Donacia* to the leaves of water-plants. In studying the development of *Donacia* Hirschler punctured each individual egg with a fine needle, whilst observing the whole cocoon under a powerful dissecting microscope. He then immersed the cocoon for two to three hours in a mixture of equal parts of 3 per cent aqueous solution of HNO_3, and concentrated aqueous solution of corrosive sublimate. The cocoons were then passed up through grades of alcohol till they had reached that of 90 per cent, in which they remained for twenty-four hours.

Then the cocoon was cut into pieces, each of which contained eight or ten eggs. These pieces were stained for twenty-four hours in a half per cent watery solution of thionin, the stain being differentiated by subsequent immersion for twenty-four hours in

96 per cent alcohol—a treatment which resulted in the embryonic area being coloured dark blue, while the rest of the egg was nearly colourless. Such eggs were then used for sections.

When it was desired to have whole mounts of the embryonic area, the chorion was carefully removed from the individual egg by means of a fine needle, the eggs were then stained for twenty-four hours in borax carmine, and differentiated for the same period in acid alcohol.

For cutting sections the fragments of the cocoon, in which all the contained eggs were parallel to one another, with the future head ends pointing in the same direction, were passed through xylol into paraffin. Wheeler mentions that when he used paraffin melting at 55°, the yolky contents of the egg took on a gummy consistency which rendered it specially suitable for cutting, and that he got perfect sections. This must be regarded as a somewhat exceptional circumstance, because yolk is usually apt to become very brittle on heating, and to break up into small fragments under the stroke of the knife, hence, usually, in dealing with yolky eggs, preliminary embedding in celloidin, as described in Chap. II., is desirable.

The egg of *Donacia*, like that of most insects, is of an elongated oval form, and the nucleus is situated near the centre, surrounded by an island of cytoplasm. When the egg is fertilized the zygote nucleus begins to divide and gives rise to many nuclei, each surrounded by its cytoplasmic island. In *Doryphora*, Wheeler got every stage from the first and was able to observe that the divisions of the daughter nuclei are at first strictly synchronous, so that in a given egg all will be in the same phase of karyokinesis. When a considerable number of nuclei have been formed some of them begin to wander outwards towards the surface of the egg whilst others remain in the interior. This wandering seems to be due to amoeboid movements on the part of the cytoplasmic islands which surround the nuclei, for these are often drawn out into comet-like shapes.

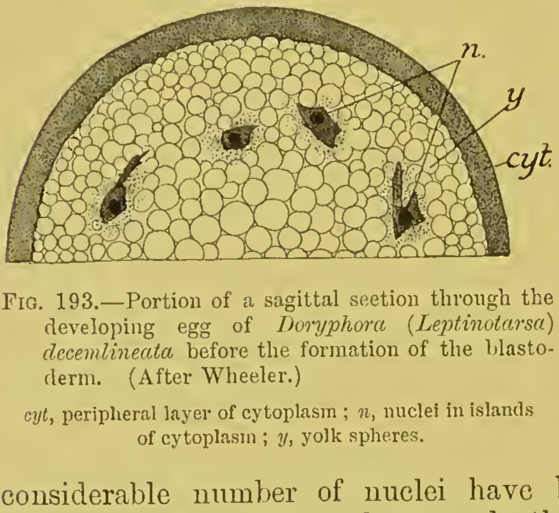


Fig. 193.—Portion of a sagittal section through the developing egg of *Doryphora* (*Leptinotarsa*) *decemlineata* before the formation of the blastoderm. (After Wheeler.)

cyt, peripheral layer of cytoplasm ; *n*, nuclei in islands of cytoplasm ; *y*, yolk spheres.

When the nuclei reach the surface they increase by further division, and eventually form a **blastoderm** consisting of a layer of columnar cells covering the whole ventral surface, and a flattened epithelium on the dorsal surface. Towards the hinder end of the egg, on the ventral surface, the columnar cells form a small mass several cells deep; this mass corresponds to the **secondary thickening** or

primitive cumulus in an Arachnid egg, and is the first rudiment of the genital organs. We arrive, then, at a stage when the egg is completely surrounded by a layer of cells, a blastoderm in fact, and when it contains in its anterior a considerable number of isolated nuclei, which become surrounded by cytoplasm and form the so-called **yolk cells.**

In *Doryphora*, according to Wheeler, the yolk subsequently segments into a number of spherical masses, each containing two or three nuclei and each surrounded by a thin layer of cytoplasm, and Wheeler regards these masses as really large yolky cells. Hirschler does not describe this process in *Donacia.*

The next change which occurs is a peculiar invagination of a portion of the dorsal blastoderm. At first this looks like a groove which is overgrown from the sides by the adjacent blastoderm, and it finally spreads out as a sheet beneath the surface of the ectoderm. This sheet degenerates and disappears; it is regarded as the "**primary dorsal organ,**" because, as we shall see, similar processes occur at a much later stage in development, and this later infolding structure is called the **secondary dorsal organ.**

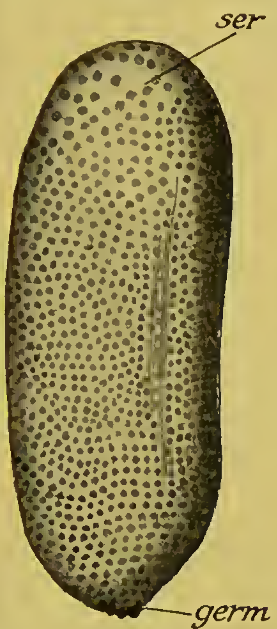


Fig. 194.—Surface view of the egg of *Donacia crassipes* at the conclusion of blastoderm formation. (After Hirschler.)

germ, primitive germ cells; *ser*, cells destined to form the serosa.

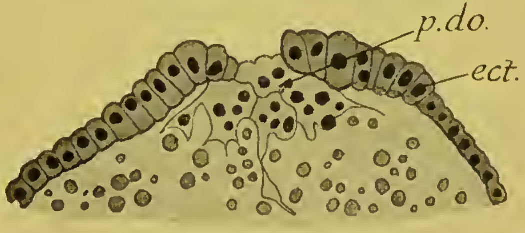


Fig. 195.—Section through the dorsal part of a developing egg of *Donacia crassipes* to show the primitive dorsal organ. (After Hirschler.)

ect, blastodermic ectoderm; *p.do*, primitive dorsal organ.

As soon as this primary infolding has taken place, the dorsal blastoderm in front of it begins to exhibit a different character from the blastoderm elsewhere; its nuclei become larger and much more widely spaced than the nuclei elsewhere (*ser*, Fig. 194). This peculiar ectoderm forms a V-shaped area, with the point directed backwards and the broad end forwards. It soon attains the anterior pole of the egg and is the rudiment, as appears later, of the outer embryonic membrane, the **serosa.** The serosa protects the embryonic area of the egg during its development, and for this reason is termed the sheath-ectoderm. It is probable that this peculiar change indicates a change in physiological function; it is suggested that the serosa ectoderm is specially suited to promote gaseous interchange between the egg and the surrounding medium.

At the same time, on the ventral side of the egg two slightly curved longitudinal folds make their appearance, and divide the

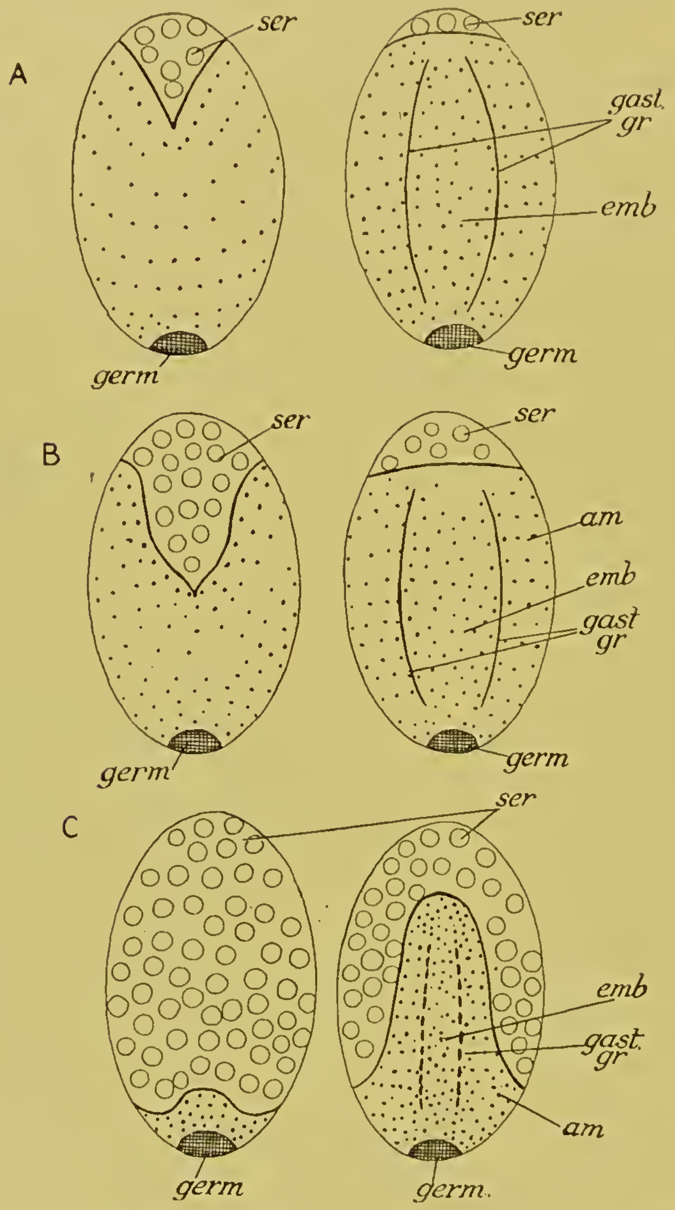


Fig. 196.—Diagrams to show the relations sustained to one another of amnion, serosa, and embryonic area in three successive stages of the development of *Donacia crassipes*. (After Hirschler.)

A, youngest stage. B, intermediate stage. C, oldest stage. In each pair of figures the left-hand one represents the ventral view, the right-hand one the dorsal view. *am*, amniotic area; *emb*, embryonic area; *gast.gr*, the limits of the gastral groove; *germ*, mass of primitive germ cells; *ser*, serosa.

blastoderm into a median and two lateral plates. The median plate gives rise to **endoderm** and **mesoderm**, whilst the lateral plates give rise to the **ectoderm.** A little later the area of the blastoderm with sparse and large nuclei is seen to have spread out, so as to completely cover the dorsal surface and also the sides of the egg, while in front it encroaches on the ventral surface; this change is effected, partly at any rate, by the modification of the cells of the blastoderm from the previous columnar to a flattened form.

All that is left of the original columnar cells is a median streak occupying the hinder part of the ventral surface, and reaching forward to about a distance of one-fourth the length of the egg from the front end. At the posterior end it curves over a little on to the dorsal surface, but between the columnar cells of the streak and the flat pale cells of the "sheath ectoderm" ordinary flat cells intervene; they form, as we shall see directly, the **amnion** or inner embryonic membrane. The streak constitutes the **germinal disc** or **embryonic area,** for on it the first organs of the embryo appear. On it are the two curved ridges alluded to above, which divide it into median and lateral plates, the last-named being very much encroached on by the extension of the modified ectoderm destined to give rise to the embryonic membrane.

Soon after this stage, at the posterior end of the egg, the fold appears which is destined to cover in most of the germinal area. This is termed the **posterior amniotic fold.** Its outer limb, termed the serosa, involves almost exclusively the sheath ectoderm; its inner limb, termed the **amnion,** is composed of ordinary blastoderm cells which pass without any break into the germinal disc or embryonic area. A small part, however, of the embryonic area itself is arched up into the amnion fold behind, and this is regarded by Hirschler as a proof that the whole of the amniotic ectoderm is

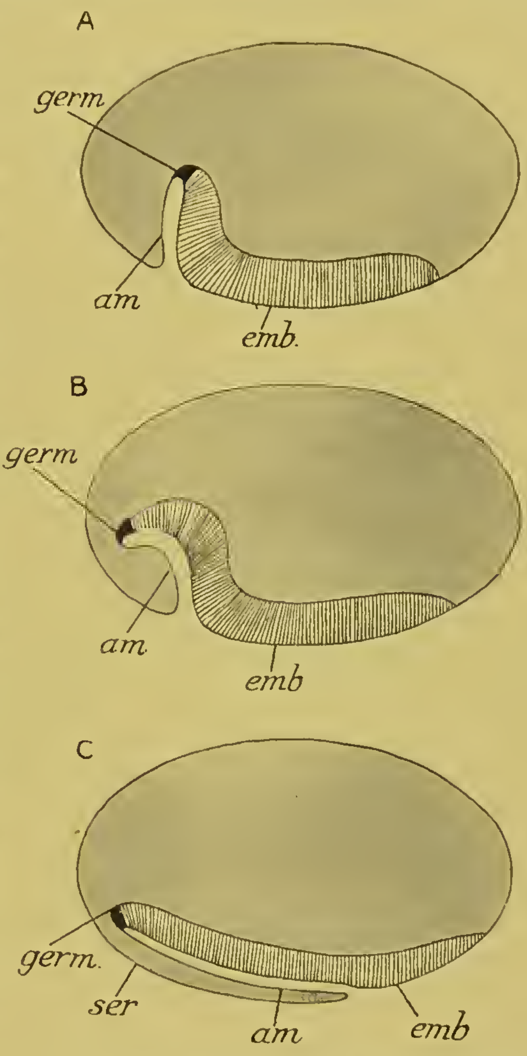


FIG. 197.—Diagrams to illustrate the formation of the posterior amniotic fold in the egg of *Donacia crassipes.* (After Hirschler.)

Letters as in previous figure. A, youngest stage. B, intermediate stage. C, oldest stage.

in reality only a secondary modification of the ordinary columnar ectoderm.

The amniotic fold is not a simple indentation at right angles to the germinal area; on the contrary, it has a peculiar trilobed growth, and the indentation is prolonged into a median and two lateral pockets. This, however, is a feature peculiar to *Donacia*, and this trilobed appearance disappears as the posterior amniotic fold advances forward over the germinal area.

Whilst this is going on, the median field of this area becomes markedly depressed beneath the surface, so as to form an elongated **gastral groove** (*gast.gr*, Fig. 198). The floor of this groove is composed of cells in a condition of active proliferation, and it forms a wedge-shaped mass in the hinder end of the groove; in the middle the groove is deepest and its cavity largest, whilst in front it is very shallow. The two sides of the groove meet, and it becomes thus completely closed off from the exterior. Throughout most of its extent this overgrowth takes place in such a way that the cavity of the groove is quite obliterated, but in the hinder region the gastral groove forms a hollow tube, which can be seen for some time lying under the ectoderm.

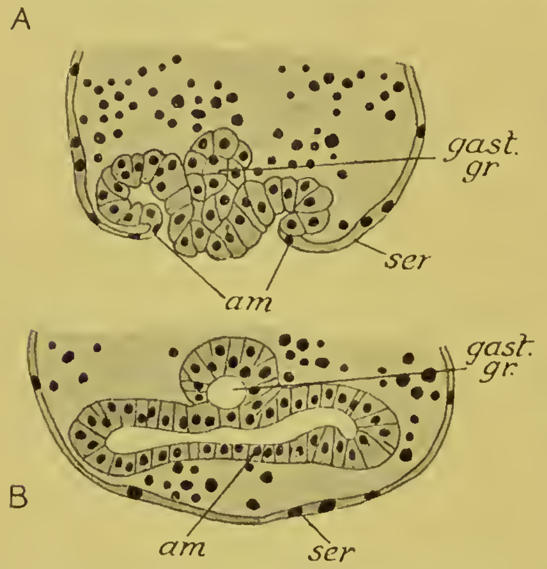


FIG. 198.—Two transverse sections through the gastral groove of the egg of *Donacia crassipes* after it is closed. (After Hirschler.)

A, section through anterior region where the amniotic folds have not yet met one another. B, section through posterior region where the amniotic fold covers the blastoderm. Letters as in previous figures. In A the gastral groove is a solid cord of cells, in B it is a tube.

At the same time also an **anterior amniotic fold** is formed, and grows backwards to meet the advancing posterior amniotic fold. The two meet and fuse, the inner or amniotic limb of one becoming continuous with the amniotic limb of the other, and the outer limb or serosa of the one joining the outer limb or serosa of the other.

Then the embryonic area begins to show the first signs of segmentation. The front end has become bilobed, and these two lobes correspond to the cephalic lobes of the crayfish. The transverse lines which indicate the division of one segment from the next, do not appear in regular order from before backward, but a few appear before the others and then the rest are, so to speak, intercalated between these. Hirschler attaches great importance to this phenomenon, and speaks of the embryo becoming at first divided into "**macrosegments,**" which subsequently are subdivided into the definitive **segments**; but as the so-called "macrosegments" do not correspond to one another in value in different insects, it does not appear that they have any morphological importance, or that the appearance of the dividing lines out of their proper order is of any more importance than the late demarcation of the chelicera segment from the procephalic lobe in Arachnida.

In *Donacia* the embryonic area is first divided into a **protocephalic** region and a so-called "**protocormic**" region, which includes all the rest of the body by a single transverse line of division. The protocormic region is then divided by two transverse lines into a "**jaw**" region, a "**thoracic**" region, and an "**abdominal**" region. In each of these regions the definitive segments are first marked off at their respective hinder borders. Thus we find a stage with two segments in the jaw region, the hinder of which is the second maxillary segment; and with two in the thoracic region also, the hinder of which is the segment of the metathorax, which later bears the third leg. This is succeeded by a stage in which the three definitive jaw segments and the three thoracic segments are clearly marked off from one another, and in which, in the abdominal region, the last three segments are clearly delimited from one another. In

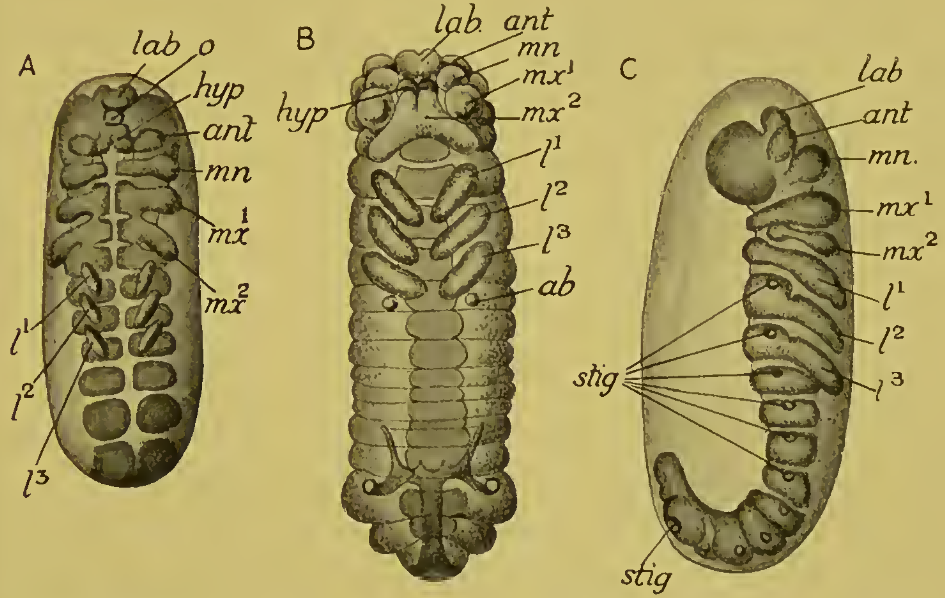


FIG. 199.—Three surface views of the embryo of *Donacia crassipes* when the germinal streak begins to show division into segments. (After Hirschler.)

A, ventral view of early stage. B, ventral view of later stage. C, lateral view of about the same stage as that represented in B. *ab*, vestigial abdominal appendage; *ant*, rudiment of antenna; *hyp*, rudiments of hypopharynx; l^{1-3}, rudiments of the three walking legs; *lab*, rudiment of labrum; *mn*, rudiment of mandible; mx^1, rudiment of first maxilla; mx^2, rudiment of second maxilla; *o*, mouth; *stig*, rudiments of stigmatae; *stig* (at the posterior end of C), the last enlarged stigma.

the protocephalic region, on the contrary, the first of the three head segments, the **acron,** is clearly marked off.

In the next stage the definitive segmentation is completely attained. The head region is divided into three segments—the **acron,** the **antennary,** and the **intercalary**; the jaw region into **mandibular, first maxillary,** and **second maxillary** segments; the thoracic region into three segments, and the abdominal region into no less than eleven segments. As soon as this definitive segmentation is attained, the appendages begin to make their appearance; first the antennae, later the three pairs of jaws, and then the three pairs of legs. All these appear as broad, slightly marked elevations, fading out into the general level of the segment near the mid-ventral line, but becoming marked towards the edges of the embryonic area.

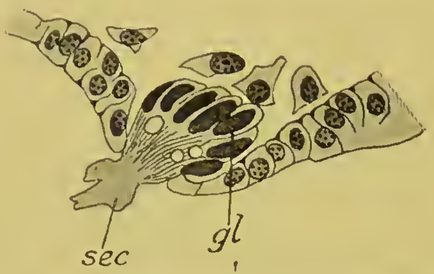


FIG. 200.—Longitudinal section through the abdominal appendage of the embryo of *Donacia crassipes* to show its glandular character. (After Hirschler.) *gl*, gland cells; *sec*, secretum.

In front of the **stomodaeum,** which has now appeared in the region of the first segment, the **labrum** appears as two broad slightly marked transverse ridges, which subsequently unite with one another. Two very small elevations appear near the middle line behind the stomodaeum, and these subsequently unite to form the apical portion of the median projection called the **hypopharynx.** The latter belong to the intercalary segment, but the basal part of the hypopharynx is formed from the sternal regions of the jaw segments. Of the abdominal segments only the first develops an appendage, which appears as a low rounded elevation on each side. Sections show that it is really a shallow cup lined with columnar cells, and that it secretes a chitonous plug, which fills up the cavity of the cup and projects on the outside. This appendage, as development proceeds, gradually disappears, the elevation sinking gradually to the general level of the segment again.

At a later stage the appendages have grown in length and certain of them undergo rotation and other changes. The antennae shift forwards so as to lie at the sides of the stomodaeum, and they are eventually situated in front of it. The axes of the jaw segments, instead of being at right angles to the long axis of the body, are inclined forwards towards the mouth, and the basal portions of the second maxillae fuse to form the **labium.** The labrum is composed of an unpaired basal piece and two distal projections, and whereas it was at first directed forwards it is now reflected backwards so as to cover the mouth. The rudiments of the legs have grown longer and indications of their division into joints have appeared. At the same time, near the mid-ventral line, at the base of each appendage, a thickening of the ectoderm is seen which is the rudiment of the corresponding ganglion.

Since the appendages of the acron which constitute the labrum are situated in the mid-ventral line between the ganglionic enlargements, Hirschler is inclined to deny their homology with the other appendages, and to suppose that they owe their origin to a secondary division of an originally unpaired outgrowth, such as gives rise to the labrum in lower insects.

We saw that, in an earlier stage, a gastral groove appeared in the mid-ventral line and became closed in as a tube. Soon all trace of a cavity in this tube disappears and the invaginated mass appears as a more or less cylindrical rod, the upper end of which is wedged into the yolk. For a while no clear boundary between it and the ectoderm can be made out, but soon the invaginated mass is sharply cut off from the ectoderm, first in the middle, then in the front part of, and lastly in the hind part of the germinal streak. When this has been accomplished the invaginated rod flattens itself out into a plate, remaining, however, thicker at the hinder end. Soon this plate becomes differentiated into a median plate, which is the rudiment of the endoderm, and two lateral plates, which are the rudiments of the mesoderm. The median plate is only one cell in thickness, whereas the lateral plates are each two cells thick; both in front and behind, however, the median plate is considerably thicker, and these regions may be termed the **anterior** and **posterior endodermic** thickenings.

Soon the mesoderm of the lateral plates begins to exhibit a moniliform structure; in a word, it is composed of thicker pieces in the centre of each segment and of thinner portions below the grooves which divide the segments from one another; that is to say the mesoderm exhibits on each side a segmentation into **somites** corresponding to the segmentation of the ectoderm, marked out by the superficial grooves dividing the segments from one another. The mesoderm of the acron, or procephalic segment, is, however, merely a flat plate which passes gradually into the thickened mesoderm of the antennary segment.

Cavities now appear in many of these **somites** and the **coelomic sacs** are thus established: they appear first in the thoracic and then in the abdominal region, but none appear in the tenth and eleventh segments of the abdomen. Still later a pair of sacs appear in the segments belonging to the second maxilla, and a pair in the region of the intercalary segment; no other cavities appear in the head or jaw regions. The thoracic sacs are small and round in section and placed laterally near the points of origin of the limbs; the abdominal sacs, on the contrary, are oval in outline and extend almost to the lateral borders of the segments.

During this time the median plate, *i.e.* the endoderm, has also been undergoing differentiation. The formation of the stomodaeum as an invagination of the embryonic area has already taken place; this is situated at the level of the acron and is oval in shape with the longer axis coinciding with the long axis of the body. About the same time a similar invagination appears at the level of the eleventh abdominal segment and is the rudiment of the **procto-**

daeum. The stomodaeal tube projects backwards and the proctodaeal tube forwards. As they grow in length they indent the layer of endoderm cells on which they impinge, and so their inner ends become clothed, so to speak, with a layer of endoderm cells. In the case of the stomodaeum this layer is continuous with the anterior endodermic thickening underneath the stomodaeum, while in its passage backwards the stomodaeum passes over it. The proctodaeal tube in its growth forwards similarly passes over the posterior endodermic thickening. The endodermic cells covering the inner blind ends of stomodaeum and proctodaeum, multiply rapidly and give rise to two lateral streaks of endoderm, right and left, in front and behind, which extend along the sides of the yolk.

Meanwhile the middle section of the original median band of endoderm has broken up into a mass of rounded cells situated in a space which becomes the median section of the **epineural sinus.** This, as we have seen, is one of the first blood spaces to be differentiated in *Peripatus*, it derives its name from the circumstance that it lies above the rudiments of the ganglia of the ventral nerve cord. The newly established lateral bands of endoderm grow towards the middle part of the embryo and here meet, so that the yolk, throughout its whole length, is covered with a layer of endoderm cells on its lateral surfaces. In subsequent development these strips grow in breadth, and eventually, about the time that the embryo hatches into a larva, the yolk is entirely surrounded by a layer of endoderm cells and the **mid-gut** is complete.

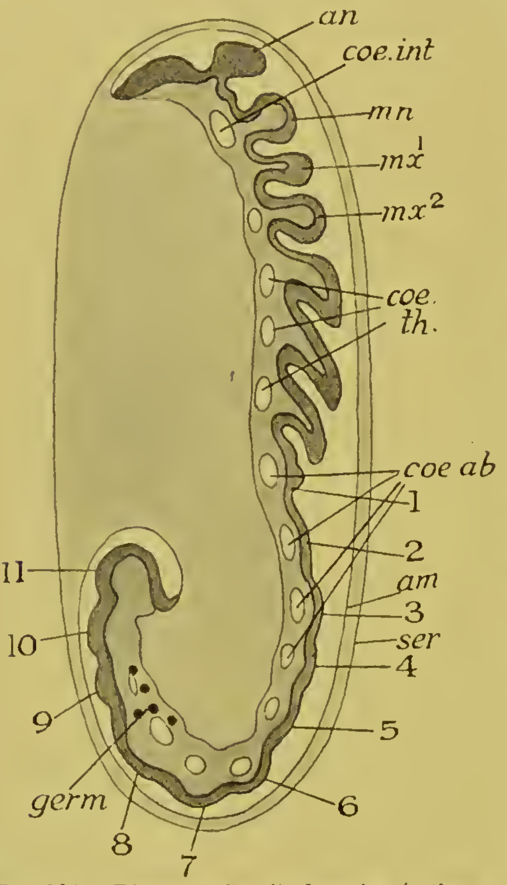


FIG. 201.—Diagram of sagittal section (rather to one side of median line) through the embryo of *Donacia crassipes* to show the coelomic sacs. (After Hirschler.)

Letters as in previous figure. In addition, *an*, rudiment of antenna; *coe.ab*, coelomic sacs of the abdominal segments; *coe.int*, coelomic sac of the intercalary segment; *coe.th*, coelomic sacs of the thoracic segments; 1-11, the abdominal segments; *germ*, primitive germ cells.

The sub-stomodaeal endodermal mass, which is situated under the stomodaeum, undergoes a strange fate: it becomes divided into right and left halves connected by a narrow bridge of endoderm, and each half becomes divided into anterior and posterior portions which assume the form of rounded masses adherent to the yolk. Cavities appear in these, and when the yolk in the mid-gut is finally absorbed a cavity appears there also. The former cavities then open into the mid-gut cavity, and it is then seen that a secretion has been developed in each rounded pocket, and that these pockets are in fact glandular outgrowths of the mid-gut. Hirschler compares these outgrowths to the liver diverticula of Arachnida and Crustacea, they are transitory structures and soon disappear in the larva (Fig. 203). The inner end of the stomodaeum is enlarged and develops a ring-like thickening of ectoderm at its inner end; in later stages endoderm cells become closely pressed against this end, and individual cells wander in amongst the ectoderm cells; thus when the cavity of the stomodaeum finally coalesces with the mid-gut it is impossible to tell where ectoderm ends and endoderm begins.

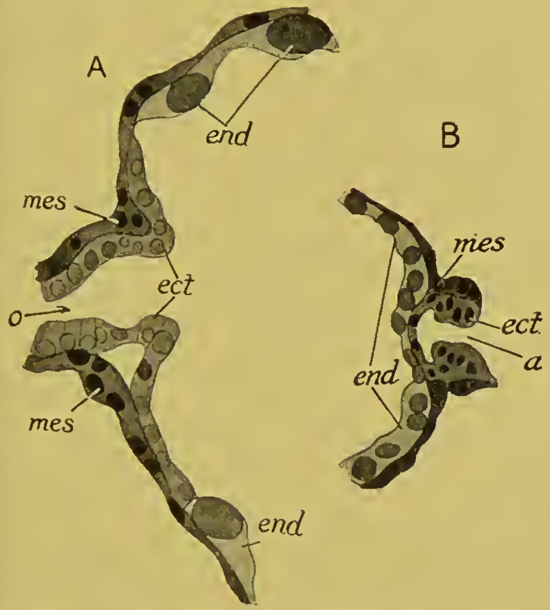


FIG. 202.—Sagittal sections through the stomodaeum and proctodaeum of *Donacia crassipes*. (After Hirschler.)

A, through stomodaeum. B, through proctodaeum. *a*, anus; *ect*, ectodermal cells; *end*, endodermal cells; *mes*, mesodermal cells; *o*, mouth.

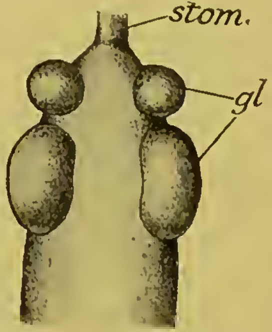


FIG. 203. — Diagrammatic representation of mid-gut of the embryo of *Donacia crassipes* showing its lateral pouches. (After Hirschler.)

gl, lateral pouches.

We must now consider the further development of the mesoderm. As the coelomic sacs increase in size they shrink away from the yolk and in this way there arises a space on each side which is the lateral portion of the **epineural sinus.** The two lateral spaces in the abdominal region, where coelomic sacs of right and left sides are in contact with one another, form from the beginning a continuous cavity; but in the thoracic region they are converted into a single cavity by the disintegration of the mid-ventral plate of endoderm described above, thus giving origin to a median sinus by the joining of those of the right and left sides.

The walls of the coelomic sac now begin to undergo differentiation. The outer wall where it abuts on the ectoderm is composed of a single layer of closely apposed cells; this is the rudiment of the body muscles. The inner wall in this region becomes converted into a mass of cells which are somewhat loosely arranged, their nuclei lose their chromatin, and fat drops are deposited in the bodies of the cells. This mass is the rudiment of the glistening white **fat body** so characteristic of insects (*f.b*, Fig. 208). Some cells of this fat body get loose in the coelomic sac and tend to choke it up. Where the outer wall of the sac passes into the inner, at its upper margin, peculiar cells called "**cardioblasts**" are differentiated; they are large cells with clear pale nuclei, and are so named because they take part in the formation of the wall of the heart.

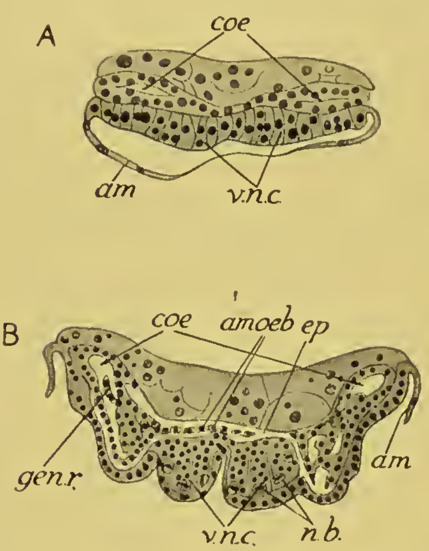


FIG. 204.—Two transverse sections through embryos of *Donacia crassipes* of different ages, to show the modifications undergone by the coelomic sacs. (After Hirschler.)

A, younger stage. B, older stage. *amoeb*, wandering blood cells; *coe*, coelomic cavity; *ep*, epineural sinus; *gen.r*, genital ridge; *n.b*, neuroblast in process of division; *v.n.c*, ventral nerve cord. (In B the section passes through a pair of ganglia.)

The inner wall of the coelomic sac clings to the yolk, and as it passes downwards it forms here a single epithelial layer which is destined to give rise later to the visceral muscles. From this layer a sheet of cells is given off which extends underneath the yolk towards the mid-ventral line, thus completing the investment of the gut with visceral muscles. Below this point the inner wall of the coelomic sac passes outwards to join the rudiment of the fat body. In that part of its course it is somewhat thickened, and is termed, for reasons to be explained later, the **genital ridge** (Fig. 204, B). As development proceeds the coelomic sacs extend farther and farther upwards at the sides of the yolk, carrying the cardioblasts on their crests.

Hirschler states that at the same time the cavities of the coelomic sacs open ventrally into the epineural sinus, owing to the breakdown of the coelomic walls at this point. This statement is supported by similar statements on the part of other authors who have worked at the development of other species of Insecta; nevertheless it is a statement which must be received with very great caution, because it is at total variance with what we know of the fate of the coelom in other groups of the animal kingdom. For this reason we doubt it, and think it is probable that the statement is a mistake, founded on a result which one is apt to get by using only paraffin wax for embedding material. When the brittle and loosely connected cells of a yolky embryo are penetrated by hot xylol and hot melted paraffin, diffusion currents are set up which are apt to produce artificial ruptures, but when the tissue is slowly infiltrated by cold solution of celloidin no violent currents are set up, and the most delicate structures are permeated and held in place. Whilst, therefore, not denying Hirschler's statement as to the communication of the coelomic cavities with the epineural sinus, we are disposed to suspend judgment on the matter till it has been reinvestigated by the aid of more refined methods. It is to be noted that the excretory tubes of *Peripatus* were confidently stated to open into the blood-spaces of the general body-cavity, till Sedgwick, by the aid of very carefully prepared sections, proved the contrary.

The coelomic sacs eventually reach the mid-dorsal line, and the cardioblasts of the two sides, which have united previously with their successors and predecessors into continuous strips of tissue, join with one another to form the tubular **heart.** Throughout the greater part of its length this union takes place at first dorsally, so that the heart tube is for some time open to the yolk on its ventral side, but in the hindermost region of the embryo the two lines of cardioblasts unite at first ventrally and then dorsally, so that in this region the dorsal wall of the heart is first formed by the ectoderm. Those portions of the outer wall of the coelomic sacs which lie immediately beneath the cardioblasts, unite with the corresponding pieces of the coelomic sacs of the opposite sides to form the **pericardial septum.** Cells budded partly from these strips and partly from the yolk form the pericardial strings of cells which lie beneath the pericardium.

The **anterior aorta,** the only definite artery which insects possess, is formed by the union in the mid-dorsal line of the two coelomic sacs which belong to the intercalary segment. The aorta becomes filled with blood cells which are derived from the loose endoderm cells at the epineural sinus, they wander up at the sides of the yolk and get between the two apposed rows of cardioblasts. Cardioblasts are not found in the last two abdominal segments.

The first trace of the **genital cells** made its appearance coincidently with the formation of the blastoderm, as a posterior thickening in that structure. When the "gastral groove" is formed this thickening separates from the overlying blastoderm and moves forwards, so that it is found later at the level of the tenth abdominal segment. Then it divides into right and left halves, and the cells of each half become to a certain extent loose from one another. This looseness is an indication that the cells composing each heap are actively migrating forwards, and a little later they are found penetrating the mesoderm in the region of the ninth segment; still later they are found as far forward as the seventh segment. When the coelomic cavities have appeared the genital cells penetrate into the "genital ridge," and in the region of the seventh segment they increase in number and form a cylindrical mass of cells, the rudiment of the genital organ.

The first beginnings of the **central nervous system** appear as two longitudinal thickenings of ectoderm, in the stage when the embryonic area is first definitely divided into segments. These thickenings start at the sides of the stomodaeum; they are indeed continuous here with thickenings in the procephalic regions which form the brain, and they continue backwards till they join one another behind the proctodaeum. Thus the central nervous system of *Donacia*, like that of *Peripatus* and of *Agelena*, may be regarded as a long drawn-out loop. The ridges are separated from one another by a shallow groove, the **neural groove,** which reaches from the hinder end of the stomodaeum back to the proctodaeum.

As the appendages make their appearance the neural ridges become segmented into thickenings lying at the bases of the appendages; these form the ganglia of the ventral nerve cord. Successive ganglia are, of course, not completely separated from one another, but are united by thinner parts of the neural ridge, which are the rudiments of the commissures. The inner parts of the ganglionic rudiments soon separate from the outer larger parts, and the inner form the definitive ganglia whilst the outermost layer forms ordinary ectoderm. In the rudiment of the ganglion are to be seen a number of rounded cells undergoing division. These are **neuroblasts** (*n.b*, Fig. 204), and the cells resulting from their division are the neurons or nerve cells.

The ectoderm lining the neural groove also becomes two layered, and the inner layer becomes separated from the outer and forms a peculiar median string of cells, which also enters into the formation of the ventral nerve cord. Each lateral thickening gives rise to a lateral group of large pale nerve cells, on the dorsal surface of which fibrillar substance appears.

The median string of cells gives rise to three smaller groups of ganglion cells, of which two arise from that portion of the string which is at the level of the ganglion, and one from the inter-ganglionic part of the median string. By the multiplication of the nerve cells, the fibrillar substance becomes completely surrounded by them.

In *Donacia* no less than twenty pairs of ganglia make their appearance, three pairs in the head, three in the jaw region, three in the thoracic region, and eleven in the abdomen. The first three, named appropriately **protocerebrum, deuterocerebrum,** and **tritocerebrum,** fuse together to form the brain; the next three unite to form the suboesophageal ganglion, and the three last ganglia of the abdomen unite to form a large abdominal ganglion.

Shortly after the appearance of the nerve ganglia the rudiments of the **tracheae** make their appearance as ectodermal invaginations, lying outside the rudiments of the ganglia and of the appendages, when these are present. Eleven pairs of these invaginations make their appearance on the last two segments of the thorax and on the first nine segments of the abdomen; they give rise to oval sacs which unite with one another in a longitudinal direction, and result in the great lateral tracheal stems of which the other trachea are outgrowths. The last pair of the tracheal rudiments are larger than the rest, and the ectoderm cells surrounding their openings **(stigmata)** are cylindrical and secrete abundant chitin, forming a horny ring from which a valve-like outgrowth projects ventrally (*stig*, Fig. 199, C).

We saw that as development proceeded the embryonic area increased in length, till by the time it is fully segmented it extends round the posterior end of the egg on to the hinder portion of the dorsal surface. This results in what is known as the **dorsal curvature.** Then the amnion and serosa become adherent to one another in the hinder region of the embryo, and a perforation is effected at this point. There follows a process closely analogous to that described as **reversion** in the embryo of the spider; that is to say, the dorsal regions of the embryo grow more quickly than the ventral, and the hinder portion of the embryo is protruded through the hole in the membranes. The adhesion of the two membranes to one another progresses rapidly forwards, and, step by step with it, the tear or rip which exposes the body of the embryo increases in extent.

The lateral portions of the embryonic area grow rapidly upwards along the yolk towards the mid-dorsal line, and, since the embryonic membranes arise from near the lateral edges of the embryonic area, the lines of origin of these membranes become shifted farther and farther upwards towards the mid-dorsal line. When the embryonic area has so far extended round the sides of the egg that its edges have nearly met in the mid-dorsal line, the remnants of amnion and serosa become invaginated into the yolk and form a tube, lying along the dorsal surface of the embryo; this tube is termed the **secondary dorsal organ.** The formation of this dorsal organ by the involution of the remnants of the amnion and serosa is progressive; it is begun behind and gradually travels forwards. As soon as the invagination at any point is complete, dissolution of the invaginated cells rapidly follows, so that at any one time the dorsal organ is of limited extent. After the completion of this organ the layer of cells of the embryonic area, which are derived from the ectoderm, completely invests the egg. Fig. 205 shows the process

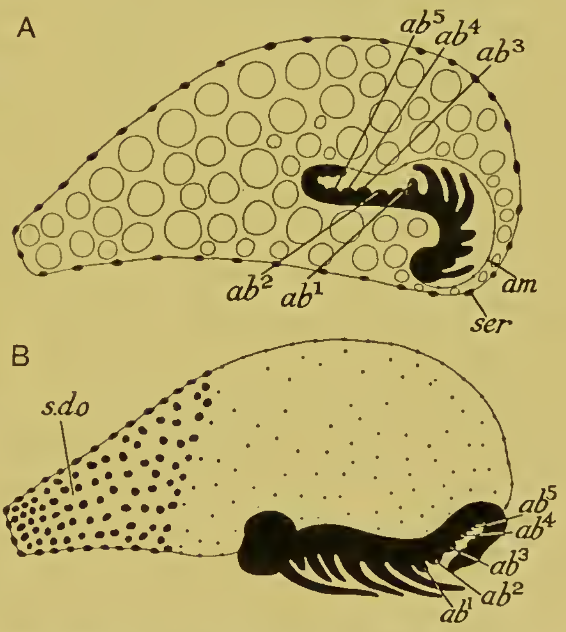


FIG. 205.—Two diagrammatic sagittal sections through the egg of *Periplaneta orientalis* in two stages of development. (After Heymons.)

A, before reversion. B, after reversion. ab^{1-5}, the abdominal appendages; *am*, amnion; *s.d.o*, secondary dorsal organ; *ser*, serosa.

of reversion in *Periplaneta,* which agrees in every detail with this process in *Donacia.*

About the same time the endoderm cells have also completely invested the yolk, and inside them the remaining yolk cells, which from the beginning of the segmentation of the egg have remained in the yolk, form an almost continuous layer parallel with the endodermal epithelium and lying on it. The yolk is progressively liquefied by the action of these cells and then absorbed. When this is complete the yolk cells degenerate, break up, and disappear.

The excretory organs of insects are known as **Malpighian tubules**; in the adult they are long thread-like tubules which open into the proctodaeum at its inner end. In *Donacia* Hirschler describes them as arising as three pairs of short sac-like outgrowths of the proctodaeum, in the stage when the embryo is still bent dorsally. When reversion takes place these sacs grow into long thread-like tubes. If Hirschler's account is correct, the Malpighian tubes are of ectodermal origin. It will be remembered that, in describing the development of the Spider, we saw in that animal the so-called Malpighian tubes arise as outgrowths of the hinder part of the mid-gut. It would therefore seem to follow that the structures termed Malpighian tubes in insects and spiders are in no sense homologous with one another.

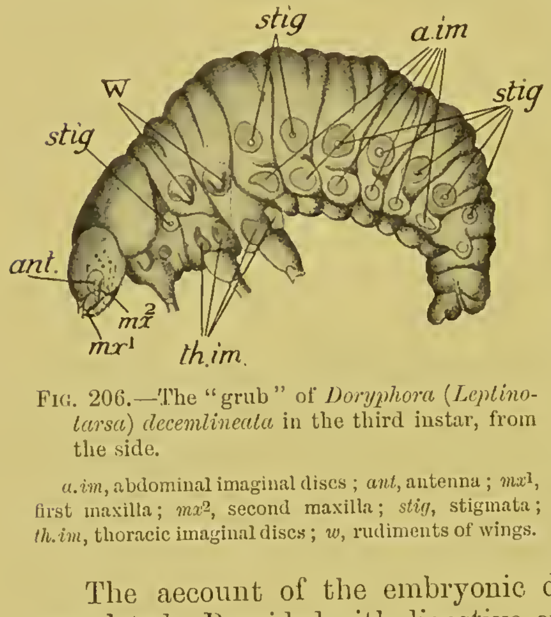


FIG. 206.—The "grub" of *Doryphora* (*Leptinotarsa*) *decemlineata* in the third instar, from the side.

a.im, abdominal imaginal discs ; *ant*, antenna ; *mx*^{1}, first maxilla ; *mx*^{2}, second maxilla ; *stig*, stigmata ; *th.im*, thoracic imaginal discs ; *w*, rudiments of wings.

The account of the embryonic development of *Donacia* is now completed. Provided with digestive, circulatory, respiratory, excretory, nervous, and muscular systems, the embryo bursts the egg-shell and emerges as the well-known "**grub,**" or larva of the beetle, which immediately begins to feed on the leaves of the plant on which it finds itself. It grows rapidly, moults several times, and then passes into a quiescent or "**pupal**" stage, during which it undergoes an extraordinary metamorphosis, and finally emerges from the pupal skin as the perfect beetle or **imago.** Of the changes which convert the grub into the beetle we have no exact account in the case of *Donacia.* For the study of this process another type must be selected.

Before entering on this study, however, we shall give a very brief survey of the most important points which have been made out in the embryonic development of other Insecta, and we shall do this in order to see how far they agree with the results which Hirschler has attained in the case of *Donacia.*

OTHER INSECTA

The account which Wheeler gives of the development of the beetle *Doryphora* bears out Hirschler's account of *Donacia* in every important point. There are, however, several interesting features in which *Doryphora* differs from *Donacia*. The embryonic area extends so far on to the dorsal surface of the egg at its posterior end that it covers two-thirds of it, consequently the embryonic area of *Doryphora* encircles the egg in a longitudinal direction almost as completely as does that of the spider. This posterior extension occurs after amnion and serosa have been formed, and as the origin of the posterior amniotic fold is forced farther and farther forward on to the dorsal surface of the egg, by the growth in length of the embryonic area, yolk passes in between its two limbs, *i.e.* between the amnion and serosa.

The serosa ultimately forms a complete envelope, its anterior and posterior folds meeting one another as they do in the Vertebrate embryo. It then separates from the egg completely and adheres to the egg-shell, consequently, when reversion begins, only the amnion is ruptured and only the remnant of the amnion forms the secondary dorsal organ. The grub of *Doryphora* has three pairs of larval eyes (and this is true of *Donacia* also); they arise as little ectodermal pits on the side of the head, in each of which the central cell is larger and clearer than the others.

But Wheeler (1889) has also studied the development of *Blatta*, a cockroach and member of the order Orthoptera, a group of insects which differ from the Coleoptera inasmuch as they emerge from the egg as perfect insects except for the want of wings.

In the case of *Blatta* the embryonic area is always confined to the ventral surface of the egg and is never as long as the egg. The mode of formation of the primary layers differs from the mode observed in *Doryphora* and *Donacia* in several important points. There is no gastral groove, but a posterior blastoporal pit, the cells lining which proliferate actively, giving rise to two streaks of cells which extend forwards and constitute the lateral bands of mesoderm. When the coelomic sacs make their appearance those in the thorax extend into the legs as in *Peripatus*, and are not confined to the bases of the leg as in *Donacia*. No trace of the endoderm can be distinguished until a much later period. When it is seen it consists of two thin bands of very small cells lying on the lateral surface of the yolk and connected with sheets of similar cells attached to the inner ends of stomodaeum and proctodaeum.

By this time, however, the mesoderm has become segmented and has developed coelomic cavities, and the walls of these have undergone differentiation.

From the similarity of the endoderm in *Blatta* at this stage to its condition at an earlier stage in *Doryphora*, Wheeler concludes that in *Blatta* also the endoderm has been derived from the cells of those bands to which the blastoporal pit gives rise.

Heymons (1894) has, however, drawn a completely contrary conclusion from his study of Orthoptera. According to him the thin sheets of cells attached to the stomodaeum and proctodaeum (*end*, Fig. 202, A and B) have arisen by the proliferation of the inner ends of those structures, and are therefore ectodermal in character; he thus draws the conclusion that the "endodermal bands" must, therefore, also have an ectodermal origin, and that for the same reason the epithelium lining the whole of the alimentary canal of the higher insects must be ectodermal in character. Finally, he supposed that the true endoderm, which must have existed in the ancestral insect, was represented in most modern Orthoptera by degenerated yolk cells.

Heymons and his pupils have sought to show that this conclusion is true for all the higher Insecta, including the Coleoptera, though they admit that in the lower Insecta a true endoderm is present.

It is the merit of Hirschler to have shown the untenability of this theory, which is at complete variance with what is known from the study of all divisions of the animal kingdom. Wherever experiment can be applied (see p. 525), it is always found that ectoderm and endoderm are physiologically differentiated, that they possess different organ-forming substances which make them functionally irreversible.

Heymons' error is a good example of the kind of trap into which embryologists may fall; he has chosen a case on which to base this theory where the endodermal rudiment becomes distinguishable only at a very late stage of development, and where its first origin is impossible to determine with accuracy. It is almost certain that he never would have propounded such a theory if he had begun with cases where the differentiation of the layers is a more simultaneous proceeding. The whole of Heymons' theory stands or falls with the assumption that the sheets of cells attached to the inner ends of stomodaeum and proctodaeum are derived from those structures, and of this he gives no proof.

The embryo of *Blatta* develops large compound eyes on the cephalic lobes before leaving the egg, but their development has not been minutely studied. *Blatta* likewise differs widely from *Doryphora* inasmuch as the embryo develops rudiments of appendages on all the segments of the abdomen (*ab*, Fig. 205); of these, the appendages of the first abdominal segment have a glandular structure as in *Doryphora*, the last pair of the appendages persist as the **anal cerci** and all the rest disappear.

The development of the genital organs in *Phyllodromia*, another genus of Orthoptera closely allied to *Blatta*, has been worked out in great detail by Heymons (1891). The original genital cells, which we may term **primitive germ cells**, and which doubtless originate at the hinder end of the embryonic area, as in *Donacia*, were first recognized as large cells with pale nuclei lying between the yolk and the unsegmented mesoderm in the abdominal region. When the division of the embryonic area into segments takes place, they are found in segments 2-7 of the abdomen, in the coelomic wall in the region of the genital ridge, as in *Donacia*. *To them are added modified coelomic cells*, which we may term **secondary germ cells**, and, together with the primary germ cells, they form a continuous mass on each side, which is the rudiment of the genital organ; they are at first found between contiguous coelomic sacs as well as in their walls. When the coelomic sacs become choked up by the development of the fat body, the uppermost section of each retains a slit-like lumen, the inner wall of which is formed partly by the genital mass, the outer by the layer of cells which gives rise to the pericardial septum. Above the genital mass the inner wall is formed by a thin sheet of cells, the so-called **filament plate** (*fil.p*, Figs. 208 and 209). Later the slit-like lumen disappears entirely, and this sheet breaks up into eight vertical threads, united above by a longitudinal thread; these are the **terminal threads** of the ovarian tubes. Soon the genital mass also becomes divided into vertical strings connected by a longitudinal piece below. The eight vertical strings form the eight **ovarian tubes** and the piece connecting them gives rise to the common **oviduct** on each side. In the larval stage there are two oviducts. When the adult condition is attained, a median oviduct is formed as an ectodermal invagination, and into this the two oviducts of the larval period open.

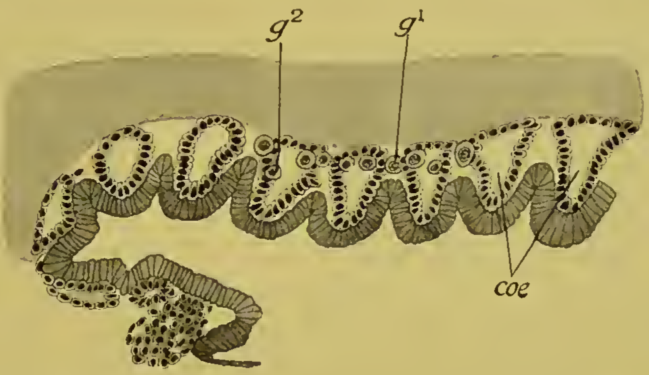


FIG. 207.—Longitudinal section through the embryonic area of an embryo of *Phyllodromia germanica*. (After Heymons.)

coe, coelomic sac ; g^1, germ cell produced before the coelom is dislodged ; g^2, germ cell formed from the wall of the coelomic sac.

When we survey the rest of the Insect world, we find that the most interesting divergences from the types we have just studied are presented by the Apterous insects whose development has been studied by Heymons (1897 and 1905). By common consent these insects are placed at the bottom of the class to which they belong.

The development of *Lepisma* shows that the early stages of segmentation, etc., resemble those of *Donacia*; there is the same multiplication of nuclei and the same migration of these nuclei to the surface. We find also an embryonic area from which nuclei are budded off into the interior, but this area is very short indeed

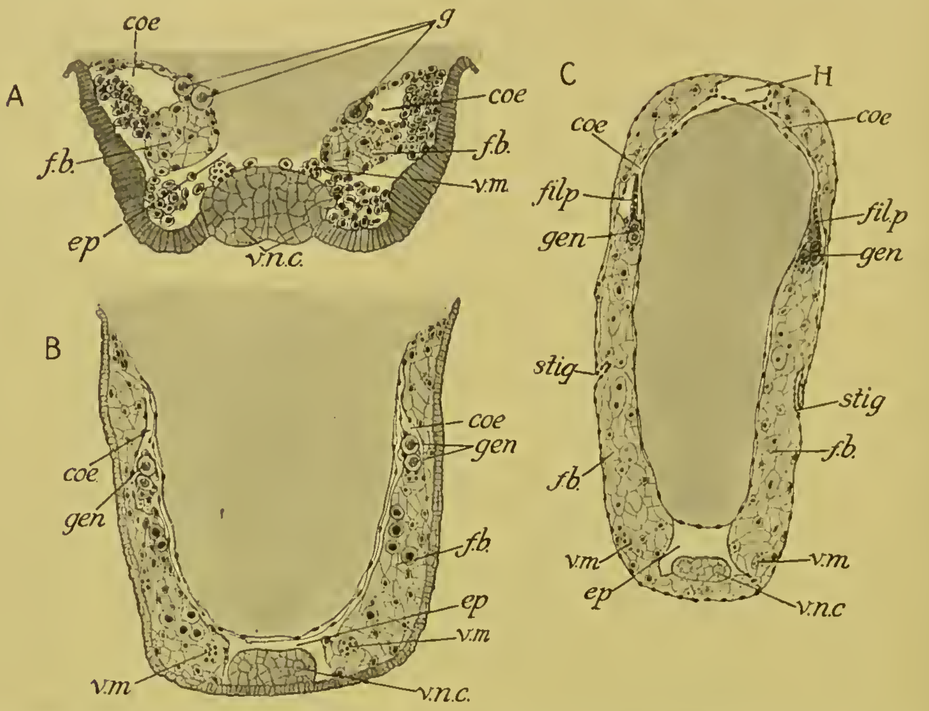


FIG. 208.—Transverse sections through three embryos of *Phyllodromia germanica* at three stages of development in order to show the development of the coelomic sacs. The fat body, and the generative organs. (After Heymons.)

A, the youngest stage. B, intermediate stage. C, oldest stage, after the completion of the dorsal surface. *coe*, coelomic sac; *ep*, epineural sinus; *f.b*, fat body; *fil.p*, terminal filament plate of the genital organ; *g*, germ cells; *gen*, accumulation of germ cells forming the genital organ; *H*, heart; *stig*, stigma of trachea; *v.m*, ventral longitudinal nerve; *v.n.c*, ventral nerve cord.

compared to the length of the egg. The cells which are budded inwards give rise to the yolk cells, and also to those cells which, at a

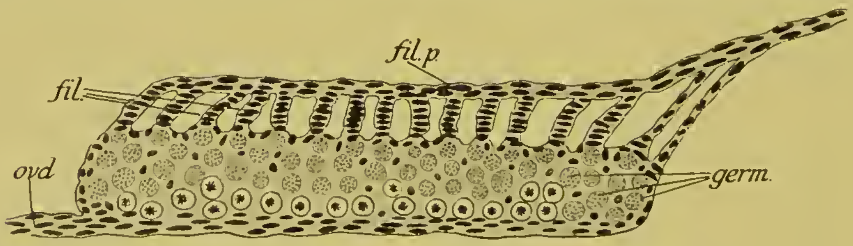


FIG. 209.—The rudiment of the female genital organs of *Phyllodromia germanica* reconstructed from sagittal sections. (After Heymons.)

fil, terminal filament; *fil.p*, plate uniting upper ends of the terminal filaments; *germ*, germ cells; *ovd*, rudiment of oviduct.

comparatively late period in development, assemble on the surface of the yolk and constitute the endoderm.

The mesodermic bands arise later by proliferation from the embryonic area, and then segmentation of the embryonic area occurs; there is, however, no gastral groove. An intercalary segment is clearly indicated. All the abdominal segments develop appendages. Of the three anal cerci which the adult possesses, the two lateral represent the appendages of the eleventh abdominal segment, the median a backward prolongation of the telson. The genital cells, as in *Donacia*, originate from a group situated in the posterior end of the embryonic area; they wander forward and are eventually found in the dorsal section of the somites.

The most interesting feature in the development of *Lepisma* concerns the formation of membranes. As the embryonic area becomes segmented it becomes bodily invaginated into the yolk, but the opening of the invagination, which we may term the **amniotic pore**, is never closed. It follows that the greater part of the ectoderm covering the egg corresponds to the **serosa**, and the nuclei of these ectodermal cells undergo the same characteristic modification as do the nuclei of the serosa cells of *Donacia*. The lateral walls of the invagination cavity, as they pass down to join the ends of the embryonic area, consist of ordinary flat cells with normal nuclei and correspond to the **amnion**.

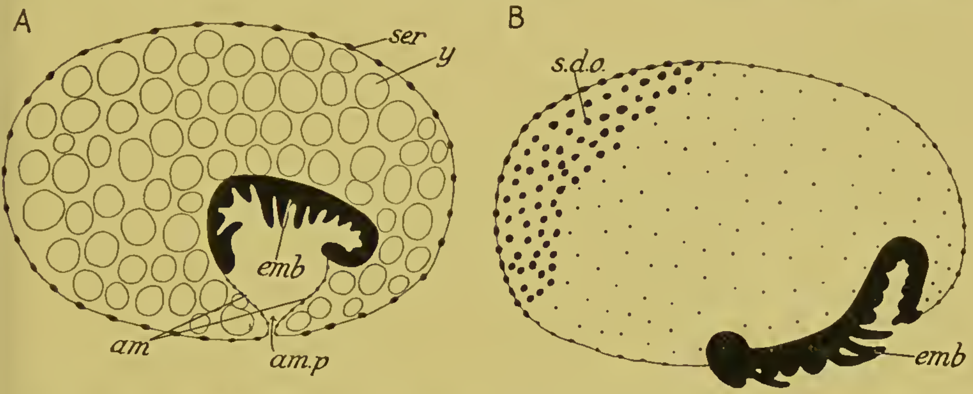


FIG. 210.—Two views of the egg *Lepisma saccharina* in different stages of development. (After Heymons.)

A, sagittal section of the stage of invagination of the embryonic area. B, external view, stage of exsertion of embryo. *am*, amnion; *am.p*, amniotic pore; *emb*, embryonic area; *ser*, serosa; *s.d.o*, secondary dorsal organ; *y*, yolk.

Turning to another representative of the Aptera, *Machilis*, which is unique amongst insects in retaining rudimentary appendages on the abdomen throughout life, we find still more primitive conditions. Here, too, the embryonic area becomes invaginated, but the invagination is not deep, and a comparatively large opening connects the invagination cavity with the exterior. The front half of the egg alone is covered with ectoderm, whose nuclei undergo the characteristic serosa modification; the hinder half remains covered with cells having ordinary nuclei, and represents the amnion. Heymons suggests that the serosa ectoderm, with its peculiar nuclei, has a distinct physiological function, possibly connected with the transfusion of gas.

The remarkable agreement in all essential details between the development of a primitive insect like *Lepisma* and a highly modified one like *Donacia*, which is disclosed by Heymons' researches, enables one to pass over the embryonic development of other groups quickly.

In Odonata (Paraneuroptera) the embryonic area is comparatively short, and the hinder end is invaginated into the interior of the egg; or to put it in another way, there is only a posterior, no anterior amniotic fold; but in all the higher insects, as in *Donacia*, there are both anterior and posterior amniotic folds, and this is the case in true Orthoptera.

In the Lepidoptera yolk spheres pass in between amnion and serosa after the front and back amnion folds have united, so that the embryonic area is said to be **immersed.** In Lepidoptera, too, all the abdominal segments develop appendages, and these are retained on those segments which, in the caterpillar larva, possess sucker feet.

In those Hymenoptera which possess a caterpillar-like larva, the same thing is true. It is true also of many Coleopterous embryos, cf. *Melolontha.* In these larvae—in early stages—all the abdominal segments have appendages, but all disappear except that on the first abdominal segment, which lasts a long time and becomes glandular.

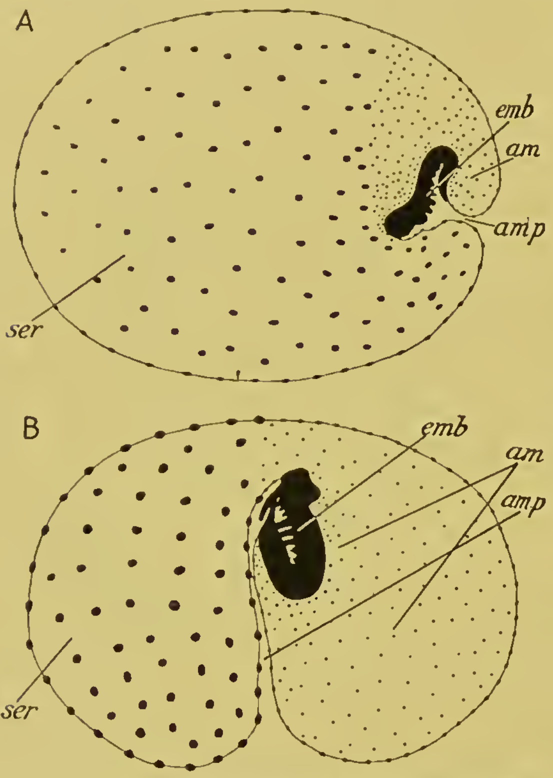


FIG. 211.—Two diagrammatic lateral views through the egg of *Machilis alternata* in different stages of development. (After Heymons.)

A, stage of incipient invagination of the embryo. B, stage of more complete invagination of the embryo. Letters as in previous figure.

The eggs of Diptera, which develop very rapidly, show a comparatively long embryonic area, and the same is true of the rapidly developing parthenogenetic eggs of Hemiptera. In fact, the variation in relative size of embryonic area and yolk seems to be the most important feature in the eggs of different kinds of insects.

METAMORPHOSIS

We shall now consider the second stage of the development of insects, viz. the metamorphosis of the larva into the adult form. The most recent and elaborate work on this subject has been done by Poyarkoff (1910). He studied the metamorphosis of a beetle, *Galerucella ulmi*, belonging to the family Chrysomelidae, *i.e.* to the family to which *Donacia* and *Doryphora* also belong. Poyarkoff used picroformol dissolved in alcohol at 60° C. to preserve the larvae, cutting them open so as to allow the fluid to penetrate.

The larva possesses nine visible abdominal segments, from which, however, all trace of appendages has vanished. The thorax bears three pairs of legs, each consisting of a basal piece and of a single joint. The head carries mandibles, two pairs of maxillae, and a single pair of rudimentary ocelli. The antennae are only represented by knobs. The stomodaeum swells out into a pharynx which is longitudinally ridged; behind this is a valve-like fold which marks the beginning of the long mid-gut. The mid-gut runs back to nearly the posterior end of the body, then turns forward till it reaches the front end of the abdomen, and then turns backward again. When it has reached as far back as the end of the first limb there is a constriction which separates it from the proctodaeum. The proctodaeum consists of a portion partly invaginated in a valve-like manner into the mid-gut, followed by a second section with six longitudinal folds which is produced into a caecal pocket: this section turns forwards. A third section bends backwards again and is suddenly constricted to form a fourth section, which is the true rectum. From the caecal pocket the four enormously long Malpighian tubules arise; they run nearly to the front end of the body, then bend back and have their terminal sections closely applied to the third section of the proctodaeum. A pair of salivary glands opens into the buccal cavity.

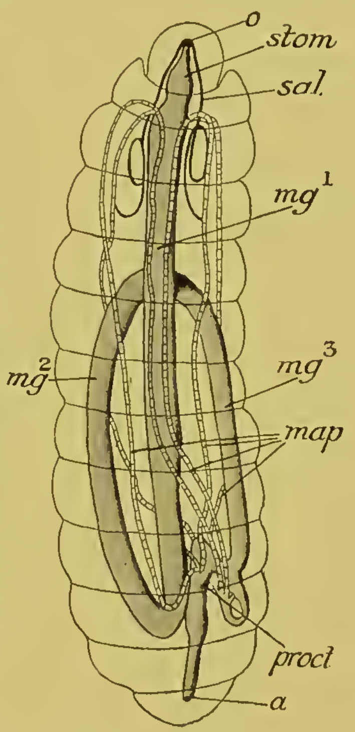


Fig. 212. — Diagram of the anatomy of the larva of *Galerucella ulmi*. (After Poyarkoff.)

a, anus; mg^1, mg^2, mg^3, the first, second, and third limbs of the mid-gut; *map*, Malpighian tubules; *o*, mouth; *proct*, proctodaeum; *sal*, salivary gland; *stom*, stomodaeum.

There are eight pairs of stigmata in the abdomen, one pair on the mesothorax and a rudimentary pair on the metathorax. There is a "brain" and twelve pair of ventral ganglia—the first being the suboesophageal ganglia. There is, of course, a dorsal tubular heart in the abdominal region. Round the bases of the antennae and of the legs there are **imaginal discs** which are slightly folded portions of the ectoderm; the cells forming these discs remain in an embryonic condition and do not secrete cuticle. On the dorsal surfaces of the second and third segments of the thorax there are pairs of similar imaginal discs which later become infolded, and from the bottoms of these discs, in the later larval life, small buds grow up which are the rudiments of the **wings**.

The larva when hatched is 1 mm. long; it moults about seven times and grows rapidly after each moult, so that when ready to metamorphose it is 8-10 mm. long. It then stops eating, since the epithelium of the mid-gut has already been cast off, and it moves more and more slowly, for the muscles moving its limbs are beginning to undergo destruction. Then it becomes bent double; it remains so for a period of twenty-four hours, and during that time the muscles of the legs are completely destroyed, and the salivary glands share the same fate. Finally, the cuticle is moulted and the larva becomes a **pupa**, which in external form and proportions resembles the adult beetle; the antennae and the legs have reached the definite proportions attained in the adult, but the wings are short and motionless. Eight or nine days afterwards a second moult occurs, and the perfect insect, or **imago**, emerges.

The principal part of Poyarkoff's investigations concern the histological changes which occur during the metamorphosis. He established the fact that, broadly speaking, the larval ectoderm becomes the adult ectoderm, but that the nucleus of each cell expels part of its chromatin, which passes to the base of the cell, surrounds itself with a ball of cytoplasm, and is then cut off from the rest of the cell. This ball lies between the bases of neighbouring ectoderm cells, and while in this position is devoured by amoebocytes.

In the case of the "tendon" ectoderm cells, in which a portion of the cell is transformed into fibres to which larval muscles are attached, this tendinous part is thrown off from the rest of the cell and is then devoured by amoebocytes like the balls derived from the other ectoderm cells. In some ectoderm cells, however, the nucleus is absorbed *in situ*, and then the whole cell degenerates and is eaten by amoebocytes. Many of the ectoderm cells are glandular; they have narrow necks and enlarged basal portions with three nuclei, and they persist in the pupa.

When the larval cuticle is thrown off the basal part of the pedicel of the gland cell grows and forms a connection with the cuticle of the pupa. There are a series of gland cells situated in the neck fold of the larva, between the head and the first segment of the thorax, each of which has several ducts; these round themselves off into balls, drop off, and are devoured by amoebocytes. Even those glands which, as we have seen, persist into the pupa stage, undergo destruction in a similar fashion when the pupa moults in order to become an imago. The glands of the adult are developed out of ordinary ectoderm cells.

The changes which occur in the alimentary canal are as follows. The first thing to be noticed at the onset of metamorphoses is an increase of the cells on both sides of the valve separating stomodaeum and intestine. The cells on the stomodaeal side elongate so as to block the cavity of the oesophagus: they cast out refractive granules, and then, at the final moult, flatten out and form the lining of the adult oesophagus. The cells on the mid-gut side of the valve multiply very rapidly and form a plug completely obliterating the lumen of the mid-gut. The old mid-gut epithelium is stripped off from its basement membrane and is devoured by amoebocytes penetrating from the body-cavity. The whole mid-gut is shortened till it is about one fourth of its former length, but Poyarkoff could not determine how this comes about. Then a new provisional epithelium is reconstituted

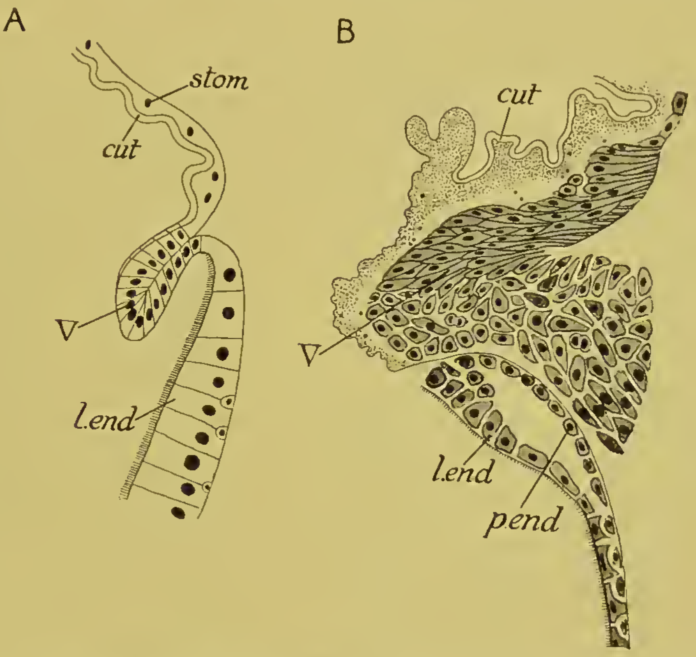


FIG. 213.—Portions of sections through the valve separating stomodaeum from mid-gut in the larva of *Galerucella ulmi*. (After Poyarkoff.)

A, in the well-developed larva. B, in the larva on the point of metamorphosis. *cut*, cuticle lining the stomodaeum; *l.end*, larval endoderm; *p.end*, pupal endoderm; *stom*, stomodaeum; *v*, valve.

from some of the cells forming the plug. This epithelium lasts during the pupal stage, at the close of which it is thrown off and the definitive epithelium of the mid-gut is formed from other cells of the plug which have hitherto remained undifferentiated.

The epithelium lining the hind-gut or proctodaeum behaves very much like that lining the stomodaeum; the cells cast out granules and then flatten, in other words the basal part of each cell *rejuvenates* itself. Then the folds in the proctodaeum flatten out and the cells become cylindrical; this shape they retain in the pupal stage, but when the adult stage is reached these cells flatten out again. The salivary glands of the larva are entirely destroyed by amoebocytes, but new salivary glands are formed in the pupa when it is three days old, appearing as a pair of ectodermic invaginations at the bases of the first maxillae.

The Malpighian tubes undergo curious changes. They are lined in the larva with large granular cells, and at intervals between these cells small **replacement cells** are seen. When the metamorphosis begins these small replacement cells multiply, become fusiform, and pass inwards till they form a string occupying the axis of the tube. The string becomes hollowed out and forms the lining of the adult Malpighian tube, whilst the cells which formed the lining in the larva gradually liquefy (Fig. 215) and their remains are removed by amoebocytes.

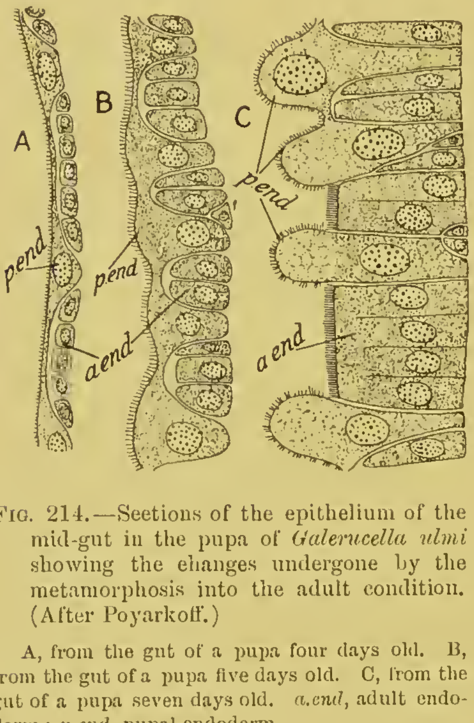


FIG. 214.—Sections of the epithelium of the mid-gut in the pupa of *Galerucella ulmi* showing the changes undergone by the metamorphosis into the adult condition. (After Poyarkoff.)

A, from the gut of a pupa four days old. B, from the gut of a pupa five days old. C, from the gut of a pupa seven days old. *a.end*, adult endoderm; *p.end*, pupal endoderm.

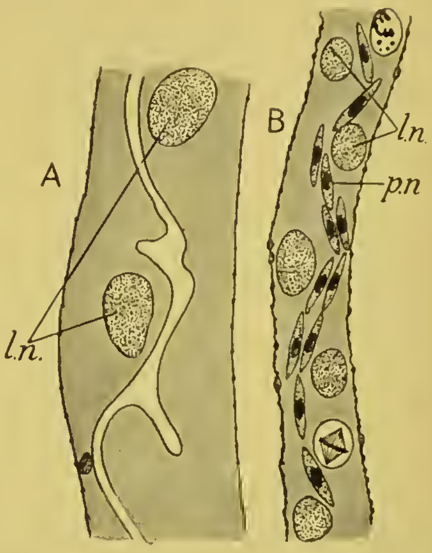


FIG. 215.—Portions of the Malpighian tubes of a larva and a pupa of *Galerucella ulmi*. (After Poyarkoff.)

A, tube of larva. B, tube of larva just beginning to pupate. *l.n*, larval nuclei; *p.n*, pupal nuclei.

Finally, many of the muscles of the larva disappear; first the striation is lost and the cytoplasm forms a homogeneous mass, then the nuclei multiply and become dispersed through it, and then the whole mass is attacked and devoured by amoebocytes. The corresponding adult muscles are formed by the growth and elongation of masses of small oval mesodermic cells which are attached to the inner sides of the corresponding "imaginal discs" (Fig. 216). In the case of some larval muscles, however, such as the powerful adductor mandibuli, a portion of the cytoplasm persists and is not eaten by amoebocytes, and its nuclei multiply and it becomes converted into the fibres of the adult muscle. The changes in the heart and in the nervous system were not examined by Poyarkoff.

Poyarkoff did not study the development of the wings in

Galerucella, but it has been carefully worked out in *Doryphora* by Tower (1909). The first traces of the wings appear before the embryo is hatched as small oval pigmented areas situated on the sides of the mesothorax and metathorax, just below the spot where, later, the tergum ends and the pleuron begins. They are therefore about the same level as the stigmata which are situated on the segments of the abdomen. When the areas thus marked out are examined by sections they are found to consist of elongated columnar ectoderm cells—distinguished from the other ectoderm cells merely by their depth and their slender character, so that they appear crowded together. In each area a circular pit appears which lengthens to form a longitudinal furrow. This furrow deepens till the whole imaginal disc consists of a longitudinal fold of ectoderm with merely a virtual cavity. The dorsal limb of this fold is much thicker than the ventral, and from this dorsal limb, during larval life, the actual wing rudiment grows out as a protuberant fold of ectoderm. The thinner ventral limb of the imaginal fold, which forms what we may term the **wing sac,** is passively stretched during the process; but not until the moult takes place which transforms the larva into a pupa do the limbs of the original fold come apart and allow the wing to protrude as an external appendage.

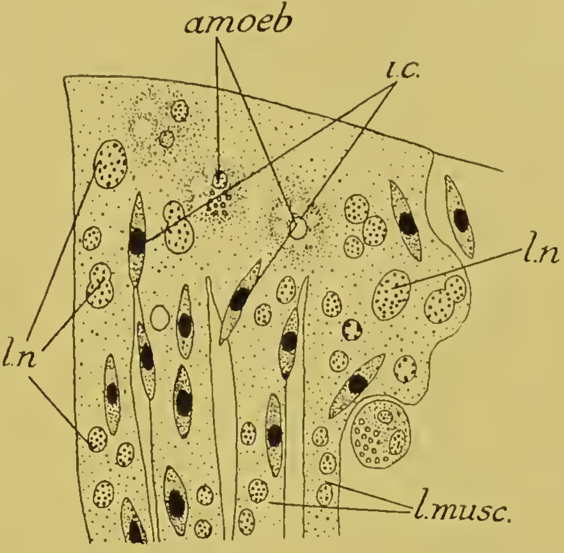


FIG. 216.—Portion of the abdominal muscles of the larva of *Galerucella ulmi* undergoing histolysis. (After Poyarkoff.)

amoeb, amoebocytes which ingest the larval muscles; *i.c*, imaginal cells, which build up adult muscle; *l.musc*, larval muscle fibres; *l.n*, larval nuclei.

The wing consists at first merely of two layers of pillar-shaped ectoderm cells each underlaid by a thin layer of mesoderm, such as everywhere underlies the ectoderm, forming a kind of dermis. The layer of mesoderm secretes a strong basement membrane on which the inner ends of these cells rest. The two basement membranes, corresponding to the two sides of the wing fold, are mostly in contact with one another, but in certain places they remain separated, leaving in this way spaces, bounded on both sides by basement membrane, some of which are in open communication with the general body-cavity.

These spaces are the primary **veins** of the wing; they become much swollen during the pupal moult, for the abdomen contracts and its contained blood is necessarily forced forwards. At the same

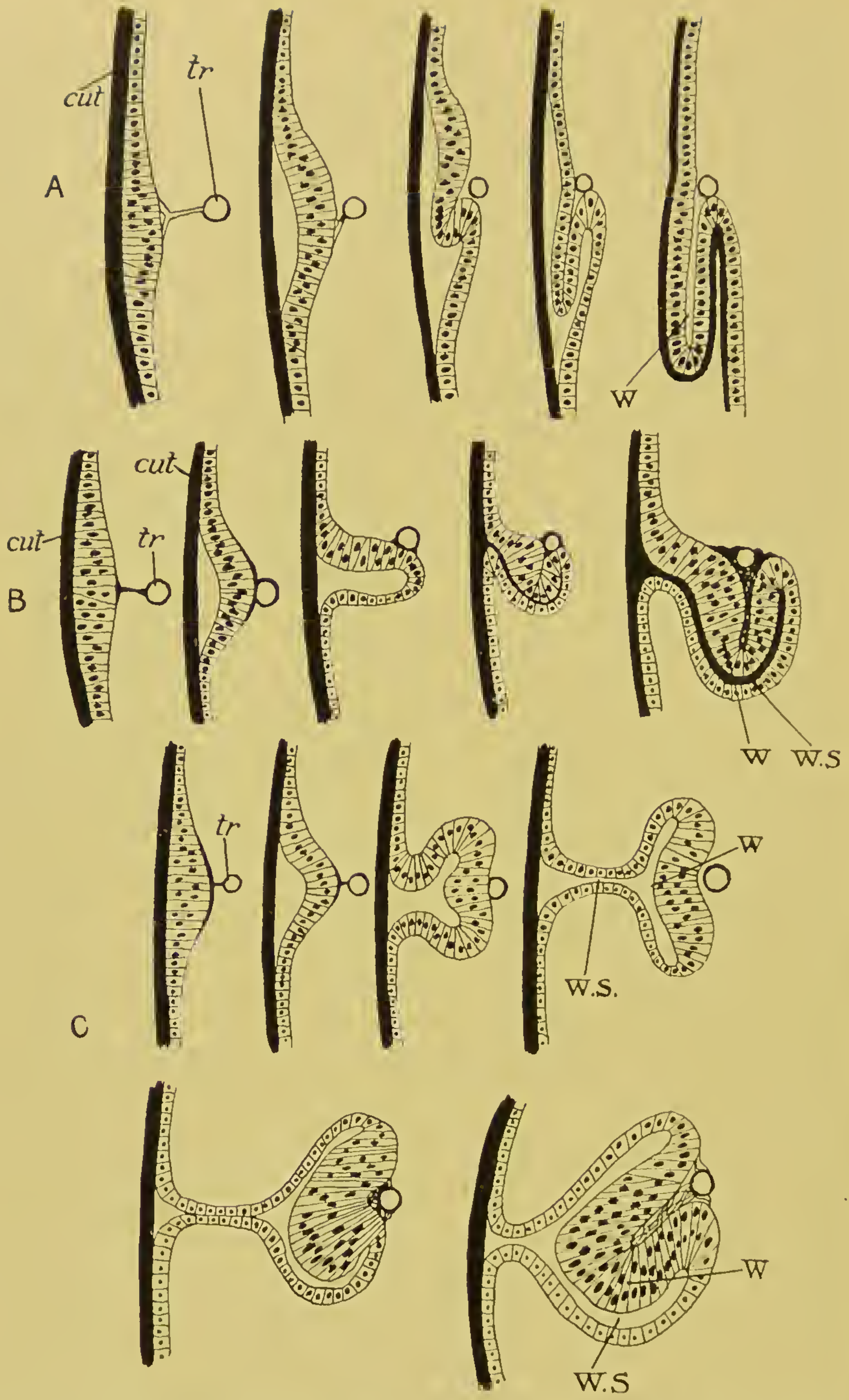


Fig. 217.—Diagrammatic sections through the imaginal disc of a wing in various stages of development, showing how the wings develop in various groups of insects. (After Tower.)

A, wing development in Orthoptera, Odonata (Paraneuroptera), Ephemeroptera, and many families of Coleoptera (cf. Cerambycidae and Buprestidae). B, wing development in Lepidoptera and in Chrysomelidae (including the genera *Donacia*, *Doryphora*, and *Galerucella* amongst Coleoptera). C, wing development amongst the higher Diptera (Muscidae). *cut*, cuticle; *tr*, trachea; *w*, rudiment of wing; *w.s*, wing-sac.

time the nuclei and cytoplasm of the ectodermal cells of the wing migrate outwards, and the basal portions of these cells contract till they form attenuated fibres attached to the basement membrane; in this way wide spaces are formed between neighbouring ectoderm cells.

In the wing rudiment of the larva there are two tracheae arising from the dorsal longitudinal trunk, which grow in along the course of the wing veins. Towards the end of the larval period these tracheae give off abundant branches, the **tracheoles**, which penetrate into the spaces between the bases of the ectoderm cells, and in each of these cellular outgrowths a delicate coiled tube develops. During the pupal stage these tracheoles seem to be absorbed by the blood, but new tracheoles are developed in the adult wing as evaginations from the tracheae. Besides tracheoles, blood and leucocytes are the only other elements which enter the wings. The muscles moving them are confined to their bases and are attached to the thorax.

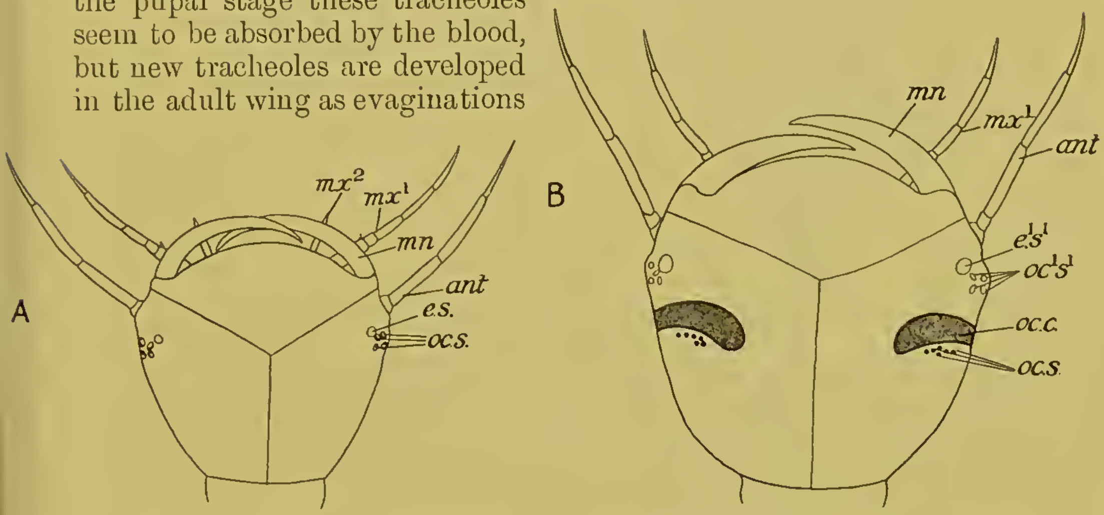


Fig. 218.—Two views of the head of the larva of *Dytiscus marginalis*, in two stages of development. (After Günther.)

A, in aquatic larva. B, in larva about to pupate. *ant*, antenna; *es*, eye-spot of the larva; e^1s^1, lens of eye-spot carried away by loosening cuticle; *mn*, mandible; mx^1, first maxilla; mx^2, second maxilla; *oc.c*, rudiment of compound eye; *oc.s*, simple eyes; oc^1s^1, lenses of the simple eyes carried away from them by the loosening of the cuticle.

The development of the larval and adult eyes has been most fully worked out by Günther (1912) in the case of *Dytiscus marginalis*, a beetle belonging to the family of water-beetles (Dytiscidae). The larva has six **ocelli** on each side of the head, and in addition a rudimentary **eye-spot.** The ocelli are arranged in a vertical ellipse, and when examined by sections each is found to constitute a slit-like pit. The cells constituting the base of the pit carry visual rods; each rod consists of two semicylindrical pieces adherent to each other, and each piece seems to consist of a mass of agglutinated delicate fibrillae. The cells lining the sides bear horizontally directed visual rods, much smaller than the basal rods but of the same character. Above these horizontal visual cells come cells bearing pigment, which form the upper part of the wall of the pit. At the edges of the pit are cells which secrete a "glass body," that is a mass of gelatinous refracting substance, whilst outside these again are the cells which secrete the thickening of the ordinary cuticle termed the lens (Fig. 219).

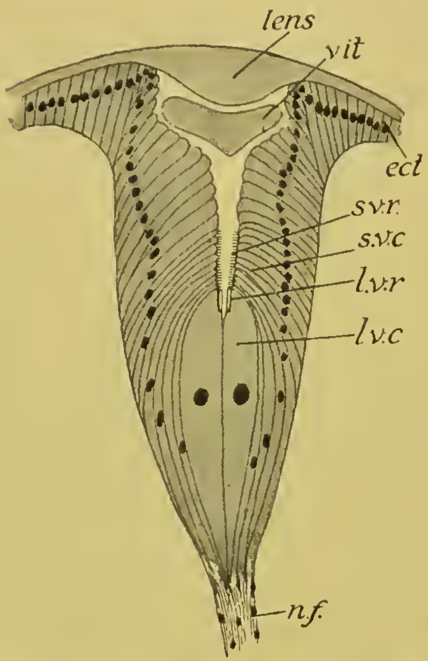


FIG. 219.—Longitudinal section through simple eye of larva of *Dytiscus marginalis* parallel to its shortest diameter. (After Günther.)

ect, ordinary ectoderm: *n.f*, nerve fibres; *l.v.c*, large visual cells; *l.v.r*, large visual rods; *s.v.c*, small visual cells; *s.v.r*, small visual rods; *vit*, vitelligeuous substance.

When the larva leaves the water and seeks a spot on land to bury itself and undergo the pupal moult, a fine pigmented line can be observed surrounding each group of ocelli like a horse-shoe. As development proceeds, this horseshoe-shaped line thickens, and the lenses of the ocelli are torn away from the deeper pigmented portions, since the lenses belong to the larval cuticle which is now being stripped off. The horse-shoe pigmented area has now increased in breadth till it has become a broad crescent, and the pigmented portions of the ocelli are close to its concave border.

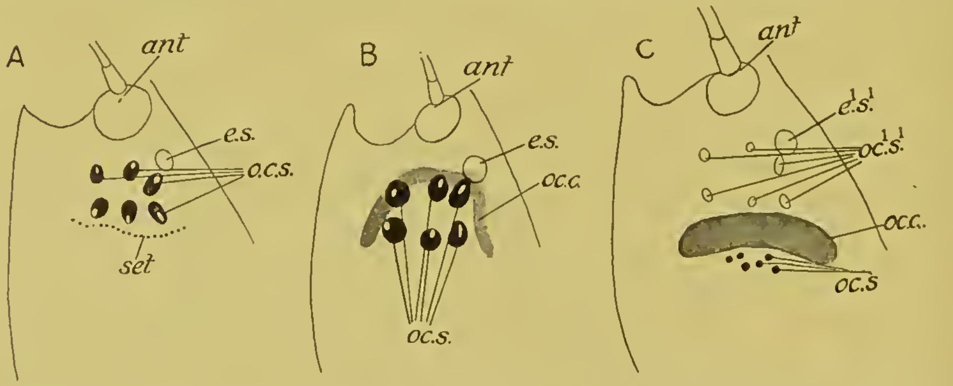


FIG. 220.—Lateral views of the head of the larva of *Dytiscus marginalis*, in three stages of development, to show the gradual growth of the rudiment of the compound eye. (After Günther.)

Letters as in Fig. 218. *set*, line of setae, marking posterior limit of eye area.

Soon, before the final moult to form the imago is completed, the remnants of the ocelli recede from the surface altogether, and the pigmented area now becomes also circular in outline, and spreads over the place the ocelli originally occupied. The remnants of the ocelli remain during life as closed pigmented vesicles attached to the optic nerves. The horseshoe-shaped pigmented area is, of course, the "**imaginal disc**" of the adult compound eye. The ectoderm here becomes thick and consists of narrow columnar cells, whose nuclei are disposed at several levels though they still constitute a single layer of cells.

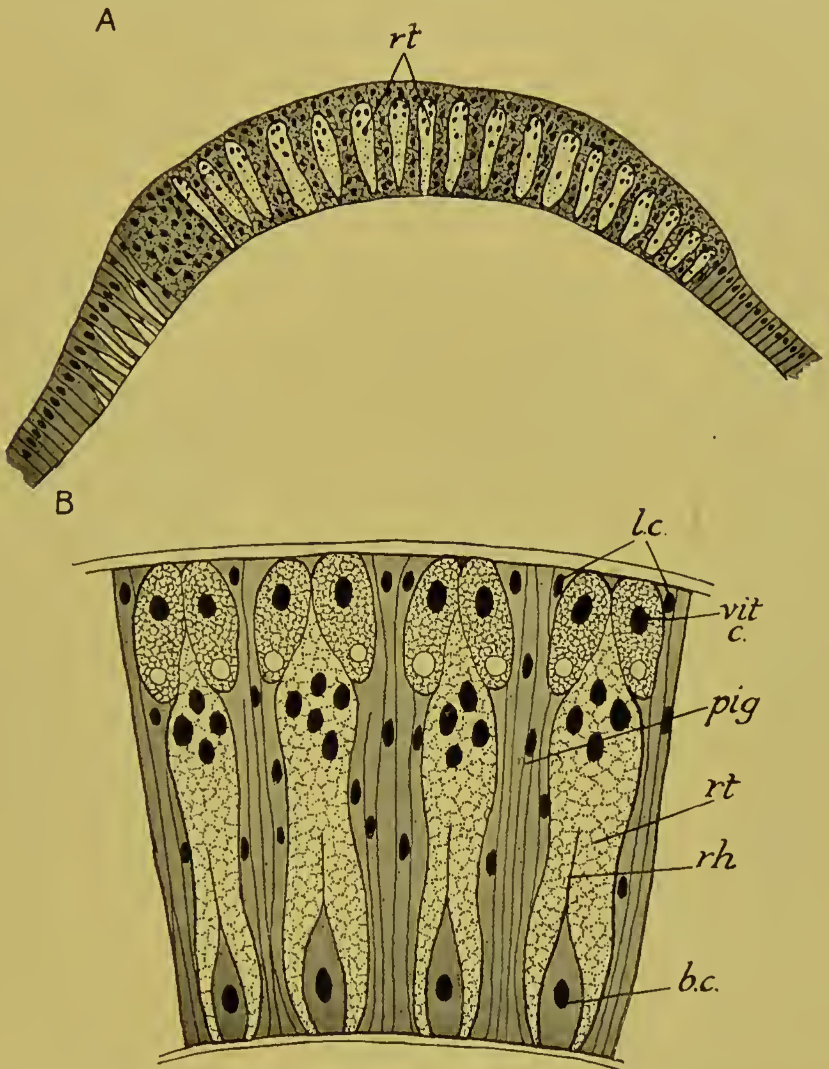


FIG. 221.—Two stages in the development of the compound eye of *Dytiscus marginalis* as seen in longitudinal section. (After Günther.)

A, in the stage of first differentiation of the retinulae. B, in the stage of crystalline cone-formation. *b.c*, basal cell; *l.c*, cells which form the lens; *pig*, pigment-secreting cell; *rh*, rhabdome *rt*, rudiment of retinula; *vit.c*, crystalline cone cell.

At one end of the horse-shoe there is a spot where the cell limits cannot be distinguished, and the ectoderm appears to consist of a thick mass with numerous nuclei scattered through it; this is the area of proliferation from which, for a considerable time, new components (**ommatidia**) are added to the compound eye. Throughout the rest of the area, certain groups of cells now become entirely retracted from the outer surface and their protoplasm becomes clearer; these are the rudiments of the **retinulae.** Each group consists of eight cells, one central and seven peripheral; one of these peripheral cells is squeezed out from between the rest, while the central cell and the remaining six peripheral cells co-operate in forming one long visual rod or **rhabdome**; the lower end of this rod originates as a thick piece in the basal cell whilst its upper part tails out to a fine point before reaching the upper limit of the retinula cells (Fig. 221).

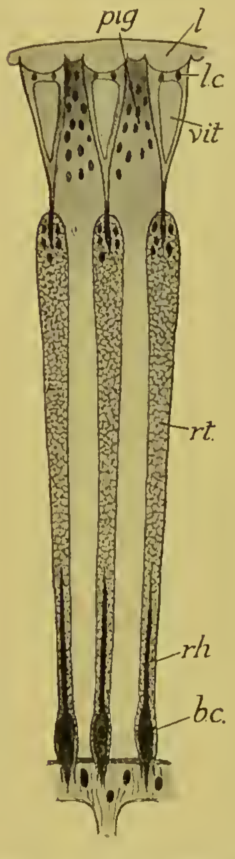


FIG. 222.—A small portion of the adult compound eye of *Dytiscus marginalis*, as seen in longitudinal section. (After Günther.)

Letters as in previous figure; *l*, lens; *vit*, crystalline cone.

Above each retinula, and intervening between it and the ectoderm, are specially modified ectoderm cells which are comparatively short, and whose bodies are filled with clear vesicles. Cross-sections show that a group of four such cells is situated above each retinula. The clear vesicles in each cell amalgamate to form a clear rod, and the four clear rods belonging to the four cells cohere to form the **crystalline cone.** The cone cells and the retinula together constitute an **ommatidium.** The cells between adjacent ommatidia extend through the entire thickness of the ectoderm and secrete pigment. The upper ends of these cells secrete the cuticle that covers the whole rudiment, but above the crystalline cone cells, where they bend to meet each other, they form a thicker area of cuticle known as the **lens.** In both ocelli and adult eye the nerve fibres of the optic nerve are basal outgrowths of the retinal cells.

It is obvious on considering the facts which have just been related that both ocelli and the ommatidia of the adult eyes are to be looked on as ectodermal pits. The ommatidium is, however, much simplified and specialized as compared with the ocellus; it possesses one large visual rod, not many small ones, and its "glass body" is formed not of an amorphous mass of secretion but of four definitely shaped fragments which cohere. The bearing of these facts will be considered when we come to treat of the phylogeny of Insecta.

For his work on the eye Günther used as preserving mixture Flemming's fluid, and also a mixture of 3 parts absolute alcohol, and 1 part glacial acetic acid. After the material was embedded the hard external cuticle was pared off by a fine scalpel, and the spot so exposed was covered with fresh paraffin.

The metamorphosis of the family Chrysomelidae, amongst Coleoptera, which we have studied in *Galerucella* and *Doryphora*, is very simple as compared to the metamorphosis of the family Muscidae in Diptera. The latter may be regarded as exhibiting the most complicated metamorphic process of all Insecta. Amongst the Coleoptera, however, which have a more prolonged period of larval existence than the Chrysomelidae, still simpler conditions are found, so that little difference is to be detected between their life-histories and those of the so-called Hemimetabolous insects, such as Orthoptera, Odonata (Paraneuroptera), and Hemiptera. Thus, according to Tower, in many Coleoptera, and in particular in the families Cerambycidae and Buprestidae, the imaginal disc for the wing consists of an area of ectoderm which shrinks away from the larval cuticle, dipping very slightly below the general level. On this area the wing develops as an *external appendage*. The imaginal discs only appear towards the close of larval life (Fig. 217, A).

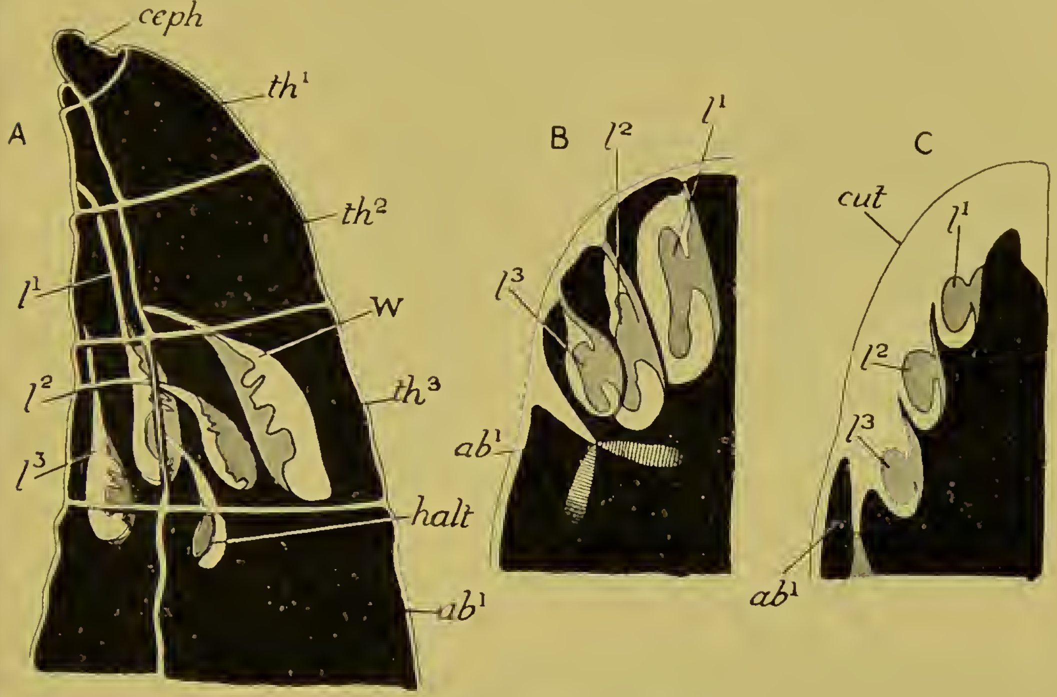


FIG. 223.—Diagrams illustrating the metamorphosis of *Musca vomitoria*. (After Van Rees.)

A, maggot with deeply embedded imaginal discs. B, early stage of pupation. C, later stage of pupation. ab^1, first abdominal segment; *ceph*, head region largely invaginated, the invaginated part represented by a white line; *cut*, cast-off cuticle; l^{1-3}, the imaginal discs for the legs, each enclosed in its sac; *halt*, imaginal disc for halteres ("balancers"); th^{1-3}, the three thoracic segments; *w*, imaginal disc for wing.

A slightly more complicated condition is found in the family Scarabaeidae where the imaginal disc, which likewise appears late, forms a deep pouch with wide opening from the bottom, out of which the wing grows up. This condition is found in the more primitive Diptera, such as Culicidae and Tipulidae, which also have long antennae. The Lepidoptera agree with the Chrysomelidae in their mode of wing development. It is obvious that the only difference between the development of a Buprestid beetle and an Orthopteran is that the Orthopteran is active at all stages of larval life till the adult condition is attained, whereas in the Buprestid a quiescent stage, the pupal condition, intervenes between larval and adult stages.

On the other hand, the development of Muscidae among Diptera, a group to which our house- and blue-bottle flies belong, shows, as has been already mentioned, very complicated conditions, which have

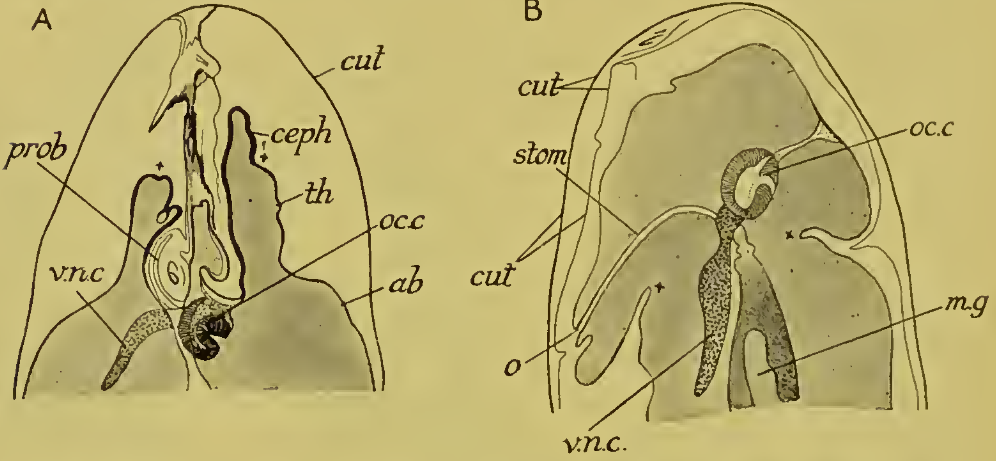


FIG. 224.—Two diagrams illustrating the metamorphosis of the head of *Musca vomitoria*. (After Van Rees.)

A, early pupa, in which the head region is still invaginated. B, later pupa, in which the head region is evaginated. *ab*, abdominal region; *cut*, cast-off cuticles of previous moults; *ceph*, head region; *m.g*, mid-gut; *o*, mouth; *oc.c*, compound eye; *prob*, rudiment of proboscis of adult; *stom*, stomodaeum; *th*, thoracic region; *v.n.c*, ventral nerve cord. The crosses mark the boundary between head region and thoracic region. In A the head region is outlined by a very thick line, the thoracic region by a line of medium thickness, the abdominal region by a very fine line.

been elucidated chiefly by the labours of Kowalevsky (1884) and Van Rees (1889). In the first place the larva is a worm-like maggot or grub, devoid not only of abdominal limbs but of visible thoracic limbs, of eyes, antennae, and jaws. The region which will form the head of the fly is represented by a pouch of ectoderm opening in front (*ceph*, Figs. 223 A, 224 A), and into this pouch, about half-way back, the mouth or stomodaeum opens.

Imaginal discs are found on the ventral surfaces of the three thoracic segments. These consist of pouches of ectoderm connected with the surface only by strings of ectoderm cells. On the segments of the abdomen both dorsally and ventrally, there are also imaginal discs, but these are just areas of thickened ectoderm which are not invaginated. Finally on the inner surface of the portion of the head pouch lying behind and above the opening of the mouth, there are two pairs of "flat" imaginal discs, the most posterior pair being the rudiments of the compound eyes, the more anterior the rudiments of the antennae. Just before the close of larval life a median imaginal disc is formed in the head pouch, in front and below the mouth, which is the rudiment of all mouth-parts and of the proboscis of the fly. From the bottoms of the imaginal "pouches," wings, the anterior functional and the posterior vestigial, forming the **balancers** or **halteres**, and the legs grow up (Fig. 223, A). As they grow the pouches enlarge and the strings of cells connecting them with the ectoderm shorten. At the same time the head pouch also shortens, its outer portion becoming evaginated so that the opening of the mouth becomes external, and the imaginal disc for the proboscis lies posterior.

Then comes the pupal stage. The imaginal pouches now open to the exterior exposing wings and legs (Fig. 223, C); the head pouch becomes completely evaginated so that the head of the fly is extruded. The dorsal imaginal pouches of the prothorax give rise to a stigma with a bunch of tracheae. The portions of the evaginated pouches surrounding the bases of the wings and legs form the adult ectoderm of the thorax. They spread out till they meet each other. The abdominal imaginal discs also extend till dorsal and ventral ones meet each other and right and left discs are united across the middle line. In this way the larval ectoderm is completely replaced. At the same time the alimentary canal undergoes profound changes. There is an imaginal ring of active proliferating cells situated at the inner ends of both stomodaeum and proctodaeum. The surface of the mid-gut has "islands" of embryonic tissue dotted over it. By the activity of these imaginal areas a completely new mid-gut epithelium is formed, as well as a new lining for stomodaeum and proctodaeum. The salivary glands have "imaginal rings" situated on their ducts where these begin to branch. New salivary glands are formed from the rings on the ducts of the larval glands.

The metamorphosis of most of the higher insects, so far as it has been studied, is intermediate in character between the types exemplified by the Chrysomelidae and the Muscid Diptera, and its real nature is brought out clearly by the researches of Verson (1905) on the transformations undergone by the alimentary canal of the silkworm moth, *Bombyx mori.* In this case the imaginal discs are disposed as in Musca—viz. there is a ring at the inner end of the stomodaeum, a similar ring at the inner end of the proctodaeum, and a number of islands scattered over the surface of the mid-gut.

Now what Verson clearly shows is that a normal cell which forms part of the wall of the stomodaeum or proctodaeum, has a limited life, and, after fulfilling its function for a time and developing its physiological peculiarities, dies and is replaced by a new cell formed by the imaginal ring, which is therefore a zone of proliferation. He points out further that the formation of both stomodaeum and proctodaeum in the embryo, is due to the existence of these zones of proliferation, and that by their activity in budding off cells external to themselves they mechanically cause their own invagination. The cells of the mid-gut likewise have a limited life—they are all eventually transformed into glandular cells, they exhaust themselves in elaborating secretion and then die and are replaced by young cells budded from the imaginal islands.

The great changes which occur at metamorphosis are due therefore to the fact that a large number of cells terminate their normal careers simultaneously, and to the fact that a large amount of proliferation from the formative zones occurs at the same time. But it is probably true that in animals of all classes, as they grow, the individual cells forming the tissues wear out and die and are replaced by young cells lying between the bases of the functional cells—at least such renewal of tissue is often observed. In some cases, as in *Galerucella*, the replacement cell may be a rejuvenated portion of the original cell, a portion of the nucleus and of the worn-out cytoplasm being cast off.

Finally, the fact that Arthropoda are enclosed in a rigid cuticle which is periodically cast off, must lead to this casting off of worn-out cells and their replacement by new ones being more or less restricted to the period of moulting—the life-rhythm of the cell tends, in fact, to be synchronous with the physiological rhythm of the production of new cuticle; and so it may be assumed we have in the metamorphosis merely an intensification of the change that goes on at every moult. Such critical moults are found in Crustacea also (see p. 198), and indeed they occur outside the limits of the phylum Arthropoda altogether, for we have every right to consider the amniotic invagination of the Echinopluteus larva to be an imaginal disc like that of *Musca*, and the destruction of larval tissue which marks the end of their larval life as quite comparable to the histolytic changes which accompany the metamorphosis of Insecta.

MYRIAPODA

Classification adopted—

Progoneata . .	**Pauropoda** **Symphyla** **Diplopoda**
Opisthogoneat . .	**Chilopoda**

Before we consider what phylogenetic lessons are to be learnt from the facts of Insect development which have been set forth in the preceding pages, we may glance at what is known of the development of those lower air-breathing Arthropoda known as Insecta Myriapoda, in which every segment of the long body bears appendages, and in which therefore regions corresponding to thorax and abdomen are not differentiated from one another.

This division constitutes, in fact, a lumber room for forms not at all closely related to one another, and it is divisible into four very distinct groups. Three of these—the Symphyla, the Diplopoda, and the Pauropoda—differ from all Insecta, and from *Peripatus* also, in having the genital openings situated in the anterior portion of the body, not far from the head. They are termed on this account Progoneata. The fourth group, the Chilopoda or Centipedes, agree with both Insecta and *Peripatus* in having the genital opening situated at the posterior end of the body just in front of the anus, and are termed on this account Opisthogoneata.

Of the development of Symphyla and Pauropoda nothing is known. A certain amount of work has been done on the development of Diplopoda or Millipedes, but the development of a Centipede, *Scolopendra*, has been worked out in great detail by Heymons (1901) and has yielded most interesting results.

SCOLOPENDRA

Scolopendra appears to be quite intermediate in its development between *Peripatus* and Insecta, as was to be expected, and there can be no doubt that in many of its features it represents a stage passed through by Insecta in their ancestral history. Heymons' main results concern (*a*) the segmentation of the egg and the formation of layers; (*b*) the segmentation of the body and the development of the appendages; (*c*) the development of the nervous system; (*d*) the development of the coelom; and (*e*) the invagination of the embryonic rudiment into the yolk, and its subsequent evagination. We shall say a few words on each of these points in order.

(*a*) In the earliest stage examined the egg shows an incomplete division of its substance into columnar peripheral segments which abut on a central unsegmented portion. This reminds one of the condition which Reichenbach found in the eggs of *Astacus*, which he called the formation of primary yolk pyramids, and which he interpreted as an incomplete segmentation of the egg. Heymons endeavours to give the same interpretation here, but there are great difficulties in the way. Thus, in the egg of *Astacus* each primary pyramid contains a nucleus in its outer portion, where it is most sharply marked off from its neighbours, but in *Scolopendra* all the nuclei are contained in the central unsegmented yolk, and it is more than doubtful if they correspond either in number or arrangement with the pyramids.

The blastoderm in *Astacus* is derived from the outermost portions of the yolk pyramids containing the nuclei, this portion being cut off from the rest of the yolk, but in *Scolopendra* the blastoderm originates as in Insecta, through the migration of nuclei from the centre to the periphery of the egg. These nuclei are surrounded by bodies of cytoplasm forming "free cells," as they may be termed, and they migrate outwards *between* the adjacent pyramids. It is clear, therefore, that the pyramids in *Scolopendra* have no relation to the division of the egg into blastomeres, but are a phenomenon of the same kind as we met with in the egg of the Spider, the peculiar yolk-rosettes of which are quite unconnected with the true segmentation.

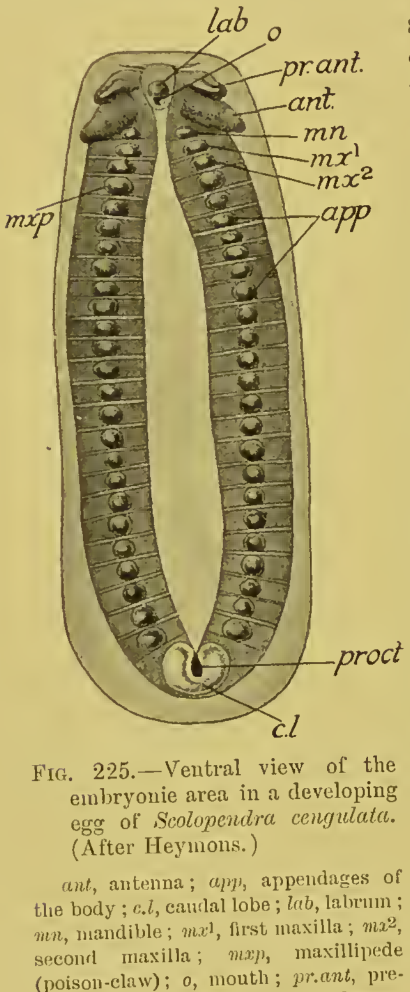


FIG. 225.—Ventral view of the embryonic area in a developing egg of *Scolopendra cingulata*. (After Heymons.)

ant, antenna; *app*, appendages of the body; *c.l*, caudal lobe; *lab*, labrum; *mn*, mandible; mx^1, first maxilla; mx^2, second maxilla; *mxp*, maxillipede (poison-claw); *o*, mouth; *pr.ant*, pre-antenna; *proct*, opening of proctodaeum.

If we eliminate the yolk pyramids the formation of the layers is almost identical in *Scolopendra* and *Lepisma*. The endoderm is budded off from a thickened area of the blastoderm termed by Heymons the **"cumulus primitivus,"** but which we might more appropriately term the **primitive streak**; the cells which ultimately form the endodermal epithelium and those that form yolk cells are indistinguishable from one another; the mesodermic bands arise from the hinder border of the cumulus and extend forwards, and their extent defines the embryonic area as in Insecta.

(*b*) The division of the body into segments and the outgrowth of the appendages therefrom occurs as in Insecta. There is an intercalary segment which, however, develops no appendages; there are three jaw segments, behind which comes the segment bearing the powerful poison claws characteristic of Chilopoda. But in

Scolopendra a segment is developed in front of the antennae on which are a pair of rudimentary appendages, the **prae-antennae** (*pr.ant*, Fig. 225). No such segment and no such appendages have been detected as yet in any other Arthropod, unless the rudimentary antennae which have been described in the embryos of certain spiders be homologous with them.

It will be remembered that Kishinouye describes a pair of coelomic sacs in the head region of *Limulus* and spiders; now the true prae-oral region or **acron** of Insecta never possess coelomic sacs. It is therefore possible that these prae-oral coelomic sacs of Arachnida may be the remnants of a lost prae-cheliceran segment corresponding to the prae-antenna.

The ectoderm covering each segment is thickened in two places on each side, one immediately above the insertion of the limb which Heymons regards as the rudiment of the tergum, and one below the insertion of the limb which he regards as the sternum. The two terga are separated by a thin dorsal membrane, and the two sterna by a thin sternal membrane. This trifid division of the dorsal covering is indicated in a later stage, when chitin has been developed, by two longitudinal furrows which mark out the dorsal sclerites into three regions, and Heymons regards it as a reminiscence of a *Trilobite ancestry* for the Centipedes, and through them for the whole of the Insecta.

(*c*) The nervous system develops as two bands of thickened ectoderm underlying the coelomic sacs and separated by a median groove. These bands are thickened in each segment, and these thickenings constitute the ganglia. In each ganglion there is an ectodermic pit, which owes its origin to the energy with which, at these spots, cells are proliferated towards the interior to constitute the nerve cells. The pits eventually become closed in and withdrawn from the surface; their outer walls do not become nerve cells but form a sheath enveloping the ganglion. From the mid-ventral membrane a string of cells is split off which, by contraction, gradually draws right and left ganglia together.

The brain consists of an **archicerebrum**, or original pair of supra-oesophageal ganglia in the acron, and of two pairs of lateral pits, a more median and a more lateral pair. These pits give rise to ganglionic masses which increase the size of the original ganglion in the acron. The small optic lobes are developed from the lateral pits. The compound ganglion so formed is known as the **protocerebrum**. The ganglia of the antennary region give rise to the **deuterocerebrum**, those of the pre-antennary segment to the bridge connecting proto- and deuterocerebrum. The ganglia of the intercalary segment give rise to the **tritocerebrum**. Since the deuterocerebrum of Crustacea resembles in its structure the deuterocerebrum of Insecta and Myriapoda Heymons concludes that the two are homologous, that consequently the antennae of Insecta correspond to the antennules of Crustacea, and that the prae-antennae of *Scolopendra* and the segment from which they spring have been entirely suppressed in Crustacea.

(*d*) The coelom is developed in the same way as in Insecta, and is eventually occluded by the transformation of the cells forming its walls into a fat-body. But before this happens each coelomic sac becomes divided by folds into three portions—a dorsal, a lateral, and a ventral. The first gives rise to the wall of the heart, the pericardial septum, and the genital tubes, as in *Peripatus* and Insecta. The lateral gives rise to muscles and to certain strings of "lymphoid tissue," and, in the region of the intercalary segment, to two masses lying at the sides of the oesophagus; these are all regarded by Heymons as the degenerate remains of "nephridia" like those of *Peripatus.* The ventral sections of the coelom, unrepresented in *Peripatus,* extend towards the middle line, and from their adjacent walls the walls of the ventral vessel are formed. In the genital segment the lumen of the coelom remains undivided and forms the proximal section of the genital duct, leading from the tubular genital organ above towards the genital opening below, as in *Peripatus.*

(*e*) The embryonic area of *Scolopendra,* marked out by the appendages and underlying somites, grows in length till it extends more than half-way round the egg. Then it is bent into a V shape by a transverse furrow, and infolded into the egg. During this process the two mesodermal bands become widely separated in the middle, meeting one another only at the anterior and posterior ends, and the yolk comes into contact with the much-stretched ventral ectoderm. Eventually, when most of the yolk has been absorbed, the embryonic area is straightened out again.

By this account we are forcibly reminded both of the early stages in the development of the spider, especially of the process called "reversion," and of the early development of *Machilis.* But there are certain great differences between *Machilis* and *Scolopendra.* In the latter, practically all the much-stretched dorsal ectoderm is eventually incorporated into the adult ectoderm or "hypodermis"; but in *Machilis* a large portion of the dorsal ectoderm undergoes the peculiar histological change described above, and forms "serosa" which is later enfolded into the yolk as the dorsal organ, it degenerates and is digested, and the dorsal "hypodermis" is largely formed by an extension upwards of the ectoderm at the sides of the embryonic area. Nevertheless, in *Scolopendra* a small portion of the dorsal ectoderm in the neck region thickens and forms a dorsal organ. The cells forming it degenerate and are absorbed, so that in it we have the first rudiment of a "serosa."

What little is known of the development of Diplopoda or Millipedes need not detain us long. We owe most of the knowledge we possess to Metschnikoff (1874), and to Heathcote (1886 and 1888). It appears that the segmentation of the egg agrees closely with that of *Scolopendra.* Metschnikoff (1874) describes a division of the surface into radial pyramids, and a conglomeration of nuclei in the central portion. The ovary of *Julus* is a wide sac beneath the gut, on the walls of which are two bands of germ cells. This sac, according to Heathcote (1888), originates by the fusion of the ventral portions of coelomic sacs, and is in fact a remnant of the coelom. The mid-gut epithelium is formed by an accumulation of yolk cells, not, as in *Scolopendra*, on the surface of the yolk, but in the form of a string traversing its centre. It follows that as development proceeds the yolk is situated, not in the cavity of the gut, but in the body-cavity, so that between Diplopoda and Chilopoda there is the same difference as between Gnathobdellidae and Rhyncobdellidae among Leeches.

The embryo is hatched as a larva, when, in addition to jaws, it has only three pairs of legs. Additional legs are added in subsequent moults. *Scolopendra*, on the contrary, is hatched with all its appendages developed, and this is true of many Centipedes which are termed on this account **Epimorpha.** But there are many Centipedes which agree with the Millipedes or Diplopoda in being hatched with an incomplete number of segments. Such forms are termed **Anamorpha.**

GENERAL CONSIDERATIONS ON THE ANCESTRAL HISTORY OF ARTHROPODA

We may now review the whole of the facts which we have learnt in order to see what light they throw on the relationships of the Insecta, Myriapoda, and Hexapoda, and the Onychophora. Since in their embryonic development most insects pass through a stage where appendages are developed on the segments of the abdomen, and since in the lowest Insects such appendages persist in a rudimentary form throughout life, there is no doubt that Insecta Hexapoda are derived from Myriapoda; and since, like *Scolopendra* and *Peripatus*, they have the genital opening situated at the hinder end of the body, they must be derived from some opisthogoneate stock like the Chilopoda, rather than from progoneate forms.

To find the origin of these last named we must go back to an ancestral condition where there were many genital openings on each side of the animal, a condition retained only in some Pantopoda, and one in which the ancestors of Myriapoda cannot have made much advance beyond that found in Polychaeta.

It is a tempting hypothesis, however, to suppose that such a state may have existed in the extinct Trilobita. In this group there was more uniformity in the character of the appendages borne by the various segments than in any other long-bodied Arthopod, and moreover they agreed with Insecta, Myriapoda, and Onychophora in possessing a single pair of antennae as prae-oral appendages.

If this hypothesis be accepted we should be dealing, in the case of Insecta, in the broad sense, with a land branch of a stock which we know to have been dominant in the seas of the Cambrian and Silurian epochs. The Onychophora would then be a somewhat degenerate offshoot from the base of this stem, degenerate in the thinness of its cuticle and in the loss of distinct joints in its appendages, primitive in most other respects, and preserved up to the present day in consequence of its secluded and burrowing habits. The Diplopoda and other Progoneates would represent another degenerate offshoot, whilst the Chilopoda and Insecta Hexapoda would constitute the main stem.

The land Arachnida may plausibly be regarded as later invaders of the land. They, too, are connected with the Trilobite stem, but more remotely, for in them a differentiation between prehensile and ambulatory legs in front and blade-like respiratory appendages behind, had been effected before the ancestral Arachnid left the water. Crustacea can also be directly traced back to primitive Trilobita; indeed the Nauplius larva, as we have already seen, is in one sense the repetition of the Trilobite stage in Crustacean ancestry.

The main difficulty connected with this hypothesis concerns the origin of the compound eye. Since *Peripatus*, Myriapoda, the lower Insects, and the larvae of the higher Insects, agree in possessing only simple pit-like ocelli, it is fairly clear that the massing together of these ocelli to form a compound eye must have occurred during the evolution of the Arthropodan stock, and had not occurred in the primitive land Arthropoda. But primitive Crustacea, primitive Arachnida, and most Trilobita possess compound eyes. The Insecta, therefore, must have been derived from primitive Trilobita in which a compound eye had not yet been developed, and, considering that a fully formed Orthopteran insect is already found in Silurian strata, this is what we might naturally expect.

One suggestion may be made here of a possible future line of research, though it must be understood it is merely an indication of such. In Trilobita, primitive Arachnida, and very many Crustacea, the compound eye is carried on a lateral projection of the prae-oral region, and in the higher Decapod Crustacea this becomes a movable appendage. In shrimps, when this appendage is cut out along with the optic ganglion it is regenerated, not as an eye, however, but as an antenna-like organ. The question is—Is it possible that in the compound eye of Trilobita, Crustacea, and Arachnida we have the representative of the prae-antennae of *Scolopendra*, and that the compound eye of Insects has had an independent origin? This is a subject which is well worth investigation.

Before leaving the subject of the Insecta, however, another very difficult problem, and one of fundamental importance for embryological science, presents itself, and that is the question—What significance are we to attach to the larval phases of Insects? We are confronted with the extraordinary circumstance that the lowest insects, like *Machilis*, *Lepisma*, etc., are hatched from the egg in practically the adult condition; that the more primitive groups of winged Insects, like Orthoptera, Odonata, etc., emerge from the egg in a condition fundamentally like the adult, except for the absence of wings; and that in the most modified Insecta a worm-like larval stage is found.

Now we have proceeded on the assumption, which all the clearest cases seem to warrant, that the larva always represents, with more or less physiological modification, an ancestral condition of the stock. Why, therefore, should the lowest branch of the Insecta fail to show this larval stage more clearly than the highest and most modified branch? Is it possible that a larval stage may be secondarily intercalated in a life-history in which the young were originally hatched with all the adult characters? Such considerations force us to look at Insect larvae a little more closely from another point of view.

Now we find that the caterpillar form of larva, with biting mouth-parts and abdominal appendages on some of the segments, crops up not only in Lepidoptera, but also in the more primitive Hymenoptera; but in the former case the appendages are borne on segments 2, 3, 4, 5, and 10 of the abdomen, and in the latter case on segments 3, 4, 5, 6, 7, 8, and 10. Again an adult form with biting mouth-parts and rudimentary abdominal appendages, which, as a matter of fact, are used to assist in locomotion, is found amongst the Thysanura, as, for instance, in *Machilis*. We conclude, therefore, that the caterpillar, or **eruciform** type of larva, represents a Thysanuran ancestor, and the reason why the Thysanura do not exhibit this phase in the earlier portion of their life-history is that it represents their adult condition.

But just as the primitive type of Echinoderm larva, found amongst Asteroidea, has undergone modification and reduction amongst the more modified groups of Ophiuroidea, Echinoidea, and Holothuroidea, so the eruciform type has undergone modification and reduction in other types of higher insects, till it becomes, in extreme cases, a legless "grub," as in Muscidae. Where the mother, in consequence of her improved powers of flight and her developed senses and instincts, is able to provide her offspring with a store of nourishment which does not require much mastication, and which ensures there shall be demand for little or no active exertion on the part of the offspring to gain food, then not only will abdominal appendages disappear, but also the thoracic appendages, and even the gnathites or jaws. In fact, we then have influences acting on the larvae similar to those which have produced legless and senseless *parasites* from adult forms in other groups of the animal kingdom.

It must be admitted that this explanation will not fit the case of Coleopterous larvae, many of which are quite active, but which do not develop abdominal appendages. There must be some independent reason for the suppression of these appendages in the larva which still may appear in the embryo (*Melolontha*), but which may be suppressed even there (*Donacia*).

The lower winged insects, often termed from the absence of a pupal stage in their development **Hemimetabola**, pass through the stage corresponding to the caterpillar, within the egg membrane; as the embryo of *Blatta*, with its well-developed abdominal appendages, abundantly proves. The so-called **nymph**, which emerges from the egg without wings, or with mere rudiments of them, corresponds to the pupal stage of higher insects, the difference being that the nymph is active and seeks its own food.

The reason why the caterpillar stage has become embryonic in the lower insects is probably closely akin to the reason why the development of the egg in an extremely primitive form like *Peripatus* takes place entirely within the maternal body. It may perhaps be expressed by a single phrase, "the pressure of modern competition." The caterpillar-like form of larva would not stand the remotest chance of survival unless the mother sought out for it a suitable environment, with plenty of food near at hand and easy to get. Where the mother has not sense enough to do this, either the stock must die out *or* the corresponding stage must be passed over within the egg membrane.

The active nymph which emerges from the egg is hard enough put to it to survive. In the case of Odonata and Ephemeroptera, to take instances, it is driven to seek shelter in an aquatic life, and may be driven to develop organs like tracheal gills, which bring oxygen to the tissues by extracting it from the water and passing it by diffusion into air-tubes deprived of external openings, and from these to the blood—a roundabout plan which betrays the secondary nature of the aquatic life of these nymphs.

In general it may be said, that since the invention of birds and bats the life of a winged insect has become a much more perilous one than formerly, and to this circumstance we attribute the fact that there is a strong tendency to shorten the life-period of the imago and prolong that of the larva. Indeed it often occurs that the imago takes no food, or, as in all Lepidoptera, only food which is incapable of prolonging life, since it is but saccharine. In the same way and for the same reason, when sex union and egg-laying are accomplished, the imago dies, no matter how securely it may be screened from enemies.

The larval stage of Insecta, therefore, agrees in its fundamental nature with the larval stages of other animals, and, we may be assured, has not been secondarily intercalated in the life-cycle: it represents a Thysanuran ancestor, but in the majority of insects it has undergone great secondary modification, and in the so-called Hemimetabola, like Orthoptera, it has become embryonic, and is passed through while within the egg membrane.

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CHAPTER IX

MOLLUSCA

Classification adopted—

- **Solenogastres**
- **Gastropoda**
  - **Isopleura** **Polyplacophora**
  - **Anisopleura**
    - **Prosobranchiata**
      - **Aspidobranchiata**
      - **Pectinibranchiata**
    - **Opisthobranchiata**
    - **Pulmonata**
- **Scaphopoda**
- **Pelecypoda (Lamellibranchiata)**
- **Cephalopoda**

One of the most surprising facts brought to light by the study of embryology is the near affinity of the Mollusca and Annelida. The adult structure in the two groups is widely different, but in their early development Mollusca and Annelida correspond almost cell for cell. The credit of having proved this important fact, by the most elaborate and laborious researches, belongs to the American School of Zoologists. Conklin's (1897) monograph on the development of *Crepidula* may be regarded as the foundation of our knowledge on this subject. A Polish zoologist, Wierzejski (1905), has, however, made the most complete study of the cell-lineage of a Mollusc as yet published. He worked on *Physa*, but *Physa* is unfortunately a form whose development is very much modified, and hence is not suitable as a type for special description. On the other hand, the common limpet, *Patella*, exhibits a most primitive type of development, and it is found all round the British and European coasts in countless numbers, and allied genera are common on the coast of America. The development of the Mediterranean species, *Patella coerulea*, has been investigated by Patten (1885) and E. B. Wilson (1904), and we therefore select it as a type for more special study.

PATELLA

There is no reason to believe that the development of the British species, *Patella vulgata*, differs in any important respect from that of *P. coerulea*, and the eggs of the British species can be artificially fertilized and the larvae reared through their complete development on a diet of the diatom *Nitschia*.

According to Wilson, the eggs of *Patella coerulea* are found ripe from March until June; the eggs of the British species, on the contrary, appear to ripen in October and November. The ripe eggs are surrounded by a "chorion"; this membrane, however, gradually dissolves when the eggs are allowed to stand in sea-water. Artificial fertilization is greatly assisted if both eggs and sperm, before being brought together, are allowed to lie in sea-water which has been rendered slightly alkaline by the addition of from 4 to 6 drops of a 5 per cent solution of caustic soda to every 500 cc. of sea-water used. In many cases more than one spermatozoon penetrates the egg and abnormal development results, but these abnormally fertilized eggs are recognizable from the fact that they divide at the first cleavage into four blastomeres instead of into two only, as normally fertilized eggs do. If, therefore, at the time of the first cleavage, the eggs which have divided into two blastomeres only are picked out with a fine pipette, a supply of normally fertilized eggs will be obtained whose further development can be studied in detail.

For his studies in cell-lineage Wilson preserved the cells simply in acetic acid. To a watch-glass full of sea-water containing the eggs a few drops of glacial acetic were added, and then, drop by drop, dilute glycerine. After a short interval, when a sufficient quantity of dilute glycerine had been added, strong glycerine was added in the same way. The eggs were in this way slowly transferred to a medium of thick glycerine, in which they were studied; they thus became absolutely transparent, whilst the cell boundaries showed up as dark lines, so that the segmenting egg looked like a glass model. A slight trace of stain with acid carmine is sometimes an improvement, but in most cases Wilson considers its use superfluous. It must be remembered that preparations made in this way are not permanent, but they afford more insight into the cell-lineage than those obtained by any other method.

The egg divides into two and then into four completely equal cells, and thus it is almost impossible to discriminate the quadrants of the egg from one another. This is quite impossible when the four first blastomeres meet quite evenly in the axis of the egg. But for four semi-fluid masses to meet in a single vertical line of junction is an impossibly unstable condition; they either meet so as to leave a vertical space between them, their inner angles being somewhat rounded off, or else they meet so as to form a "cross furrow" at one or both poles. By a cross furrow is meant the meeting of two of the blastomeres along a short surface so as to separate the other two from each other. If a given two, say B and D, meet thus at the vegetative pole of the egg, then the other two, A and C, will similarly meet at the animal pole of the egg, so that two short "cross furrows" at right angles to each other are thus produced.

Now, as a result of the study of eggs with spiral cleavage belonging to many different species, it has been found that in all cases of normal cleavage—*i.e.* where D is the left posterior macromere—the cross furrow at the vegetable pole slants upwards from left to right when looked at from above, the macromeres being placed in their normal position. But we can determine what is their normal position from the fact that the polar bodies are given off from the animal pole, and if we then look at the egg from this pole and rotate it until the cross furrow takes up the proper position, we know that the left anterior blastomere is A, the right anterior B, and so on (cf. Fig. 226). The cross furrow does not always appear in the early stages in *Patella*, and when it does not, it is impossible to be sure of the orientation of the egg.

The 8-cell stage is reached as usual by the fourth cleavage, and in it the four upper cells or the first quartette of micromeres are decidedly smaller than their lower sisters the macromeres (Fig. 226). The micromeres are separated from the macromeres as usual by the formation of dexiotropic spindles.

At the next cleavage the first-formed micromeres divide by laeotropic spindles into a set of cells, $1q^1$, above, and a set, $1q^2$, below. The cells denoted by the symbol $1q^2$ are slightly larger than those named $1q^1$. At the same time the macromeres also divide laeotropically, giving rise above to the second quartette of micromeres. These micromeres are larger than the first quartette, but still much smaller than the residual macromeres 2A, 2B, 2C, and 2D.

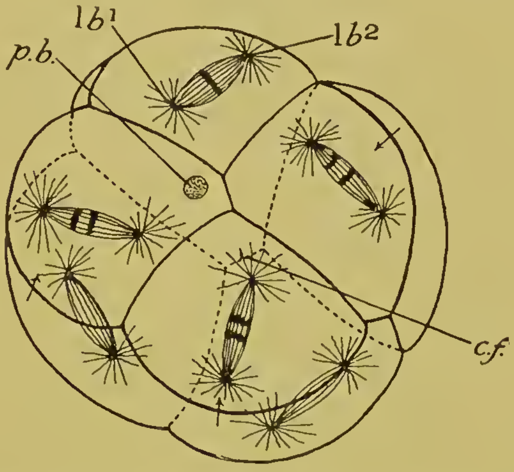

FIG. 226.—Development of *Patella coerulea.* 8-cell stage showing the spindles preparatory to the formation of the 16-cell stage. The spindles in the upper cells show the laeotropic twist. (After Wilson.)

c.f, cross furrow at the vegetable pole; *p.b*, polar bodies.

At the next cleavage the 32-cell stage is attained. Each of the basal cells 2A, 2B, 2C, and 2D divides dexiotropically so as to give rise above to a small daughter cell. These four smaller cells constitute the third quartette of micromeres, and, as in Annelida, the three quartettes give rise to all the ectoderm. The second quartette cells divide, at the same time, each into two almost equal daughter cells. The lower daughter cells derived from the first quartette $1q^2$ divide quite equally into upper cells $1q^{21}$ and lower $1q^{22}$. As in the eggs of *Polygordius*, all the descendants of $1q^2$ enter into the formation of an equatorial band of ciliated cells—or **prototrochal girdle**, which later encircles the larva about its equator. The uppermost cells of the egg, $1q^1$, divide into larger daughters, $1q^{12}$, below and very small daughters, $1q^{11}$, above, which occupy the extreme animal pole of the egg.

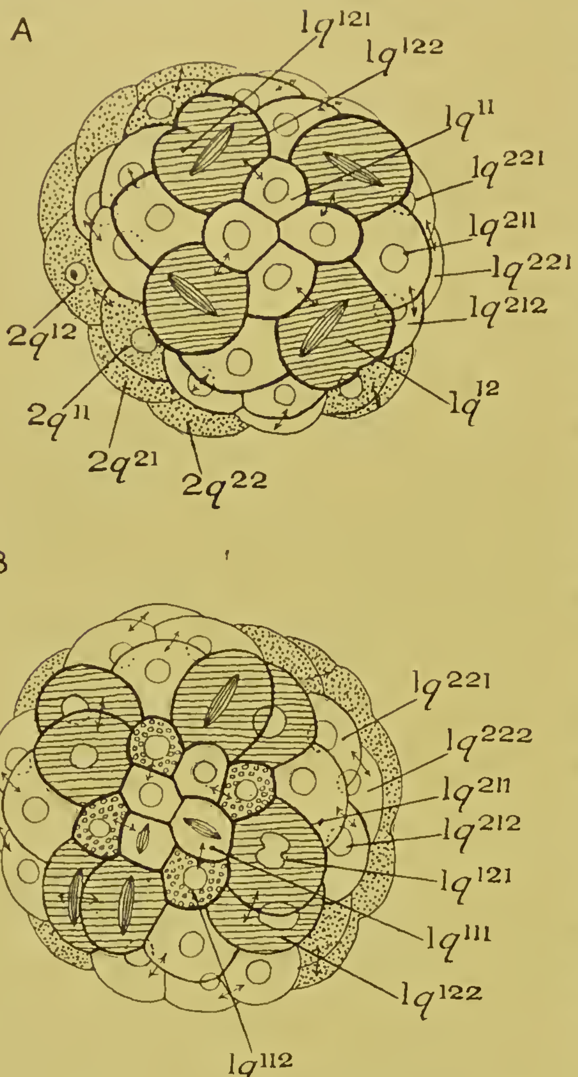

FIG. 227.—Two stages in the development of the upper hemisphere of the embryo of *Patella coerulea* viewed from above. The primary trochoblasts are unshaded. The cells belonging to the second quartette are dotted, those forming the Molluscan cross are ruled with horizontal lines, whilst those forming the Annelidan cross are covered with small circles. The apical cells are unshaded. (After Wilson, somewhat altered.)

A, stage in which the cells forming the Molluscan cross are undergoing their first division. B, stage in which the cells forming the Molluscan cross are dividing for the second time, and in which the cells forming the Annelidan cross have appeared.

The egg up till now is perfectly radially symmetrical, and no difference whatever can be detected between its different quadrants in this respect. With the sixth cleavage, however, leading to the formation of the 64-cell stage, this radial symmetry is lost, and is replaced by a bilaterally symmetrical arrangement.

The divisions which constitute this cleavage are as follows. The cells $1q^{11}$ divide in regular spiral order into cells $1q^{111}$, which correspond to the **apical cells** of *Polygordius*, and into $1q^{112}$, the **peripheral rosette cells**, which correspond to the **Annelidan cross** in the larva of *Polygordius*. The cells $1q^{12}$ also divide spirally into daughters of equal size, and give rise to the so-called **Molluscan cross**, which at this stage has curved arms. The upper daughters, $1q^{121}$, form what are called the **basal cells** of the cross; the lower daughters, $1q^{122}$, constitute the **intermediate cells** of the cross, but the tips of the four arms are constituted by the four **tip cells**, $2q^{11}$, which belong to the second quartette of micromeres. The cells $1q^{21}$

and $1q^{22}$ also divide into equal daughters, and give rise, as in the larva of *Polygordius*, to four lozenge-shaped groups of four cells. These sixteen cells are termed the **primary trochoblasts** (Fig. 227, $1q^{211}$, $1q^{212}$, $1q^{221}$, $1q^{222}$).

The cells of the second quartette of micromeres divide as follows: $2q^1$ divides into the tip cells $2q^{11}$, which alternate with the four groups of primary trochoblasts, and into four lower cells, $2q^{12}$. The cells $2q^2$ divide into four larger upper cells, $2q^{21}$, and four smaller lower cells, $2q^{22}$, situated near the vegetative pole of the egg. The cells $2q^{21}$ are situated nearly side by side with $2q^{12}$.

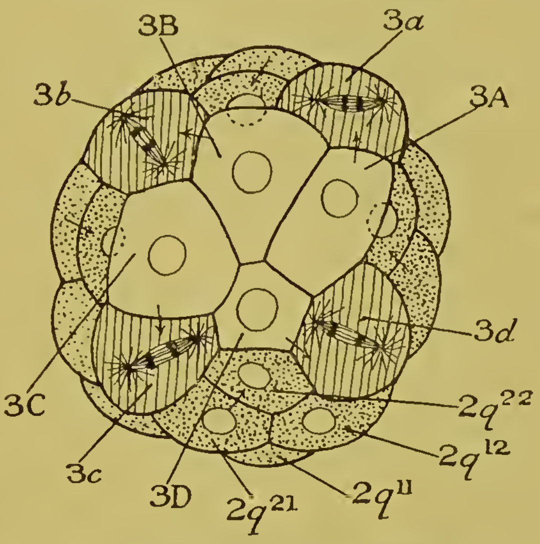

Fig. 228. — Stage in the development of the embryo of *Patella coerulea*, showing the first division of the third quartette and the beginning of bilateral symmetry seen in the direction of the spindles in 3c and 3d. Viewed from the vegetative pole of the egg. The apparent small size of the macromere 3D is due to its movement inwards, which is the beginning of the process of gastrulation. The cells of the third quartette are ruled with vertical lines. Those of the second quartette are dotted. The residual macromeres are white. (After Wilson, somewhat altered.)

Of the cells of the third quartette 3a and 3b divide spirally like all the cells we have so far mentioned, giving rise to $3a^1$ and $3a^2$, $3b^1$ and $3b^2$, respectively; but 3c and 3d divide by spindles so directed as *to converge towards the median plane of the embryo and make equal angles with it; and this division constitutes the first appearance of bilateral symmetry in the egg* (Fig. 228), which has now assumed the form of a hollow **blastula**.

The residual macromere 3D glides upwards into the **blastocoele**, remaining for a time in connection with the surface by a narrow neck; it then divides into an internal cell, 4D, which will form part of the mid-gut, and into a superficial cell, 4d, which is the **mother cell of the mesoderm**. This gliding upwards of 3D, which we can only attribute to altered cytotaxis, is the first sign of the **process of gastrulation** (Fig. 229). The other macromeres, 3A, 3B, and 3C, then, a little afterwards, follow the example of 3D and migrate inwards, also remaining for a short time in connection with the surface by long necks. Then they also divide; but, in the case of each of these, both their daughters form part of the wall of the mid-gut.

As development proceeds further, divisions take place amongst the apical cells, in the rosette cells, and in the cells of the Molluscan cross. The cells of the Annelidan cross divide only once more.

The division of the apical cells $1q^{111}$ results in the formation of a well-marked plate of eight central cells at the animal pole (Fig. 231).

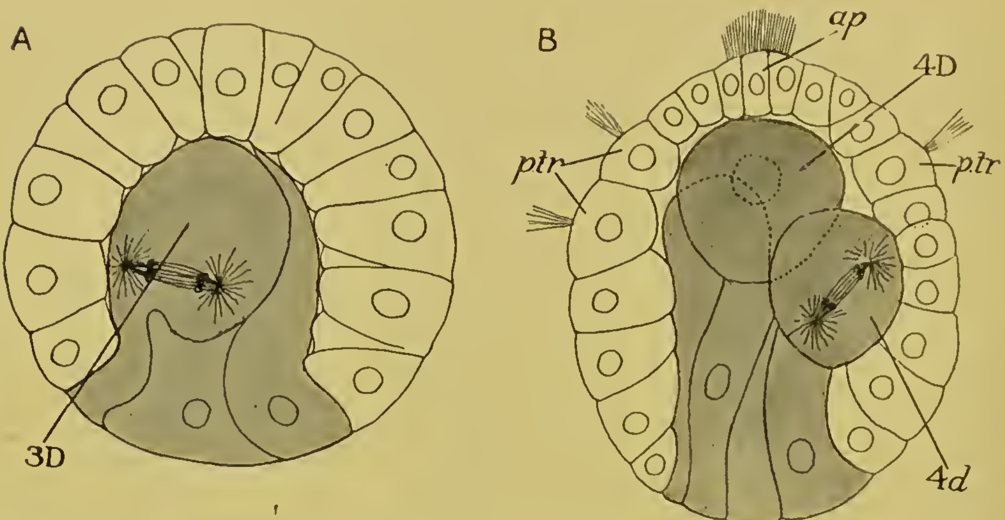

FIG. 229.—Two stages in the gastrulation of *Patella coerulea* in optical longitudinal section. The residual macromeres are shaded. (After Wilson.)

A, stage showing the formation of the mesoderm mother cell, 4*d*, and the inward migration of the macromere, 3D. B, stage showing the division of the left mesoderm mother cell, and the inward migration of the other macromeres. *ap*, apical plate; *p.tr*, prototroch.

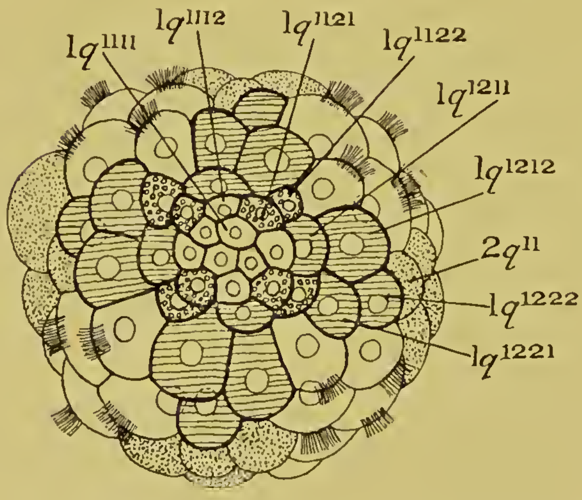

FIG. 230.—The "Ctenophore" stage in the development of *Patella coerulea*. Seen from above. The cells are marked as in Fig. 227. (After Wilson, somewhat altered.)

Meanwhile other changes take place. The four groups of primary trochoblasts develop transverse rows of powerful cilia, each row bearing a striking resemblance to a single "**comb**" of a Ctenophore, and the apical cells develop a terminal tuft of long stiff cilia. The embryo at this stage, when looked at from above (Fig. 230), bears a striking resemblance to a larval Ctenophore; and this stage, which is reached eight hours after fertilization, is termed by Wilson the **Ctenophore stage.**

The cells constituting the apical plate do not appear to divide further, but the cells forming the arms of the Molluscan cross undergo repeated divisions, in consequence of which these arms cease to be distinguishable from one another, all four constituting a mass of small cells covering the upper hemisphere of the egg (Fig. 231).

The ectodermic cap of micromeres extends downwards so as almost to reach the vegetative pole by the process of growth known as **epibole** (see p. 72), and thus, by **epibole**, the process of gastrulation is completed. For a time a small opening persists at the vegetative pole, where the macromeres are uncovered by micromeres, and this is the **blastopore.**

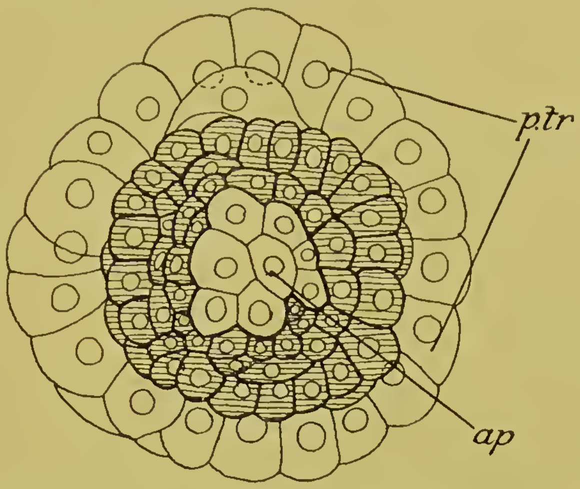

Fig. 231.—View of the upper hemisphere of an embryo of *Patella coerulea* just before hatching. (After Wilson.)

ap, apical cells; *p.tr*, prototrochal cells.

In the meantime the mother cell of the mesoderm (4d) has become divided into right and left daughters. The prototrochal girdle has now acquired the form of a complete circle through the acquisition of cilia by those cells which intervene between adjacent groups of primary trochoblasts. These cells may be termed **secondary trochoblasts,** and two of them in each quadrant are derived from the first quartette; they are in fact daughters of the outer cross cells $1q^{122}$. One secondary trochoblast in each quadrant is derived from the second quartette; these are the **tip cells** of the arms of the Molluscan cross, $2q^{11}$: and in this way the number of cells entering the prototrochal girdle is raised by three in each quadrant, making a total of 28 (16 + 12). Occasionally the tip cell divides into two, and then there are 32 secondary trochoblasts. The cilia borne by the secondary trochoblasts are at first much shorter than those borne by the primary trochoblasts (Fig. 232). By secondary shiftings these cells become rearranged so as to constitute a complete girdle of powerful cilia, behind which is an equally complete girdle of smaller cilia.

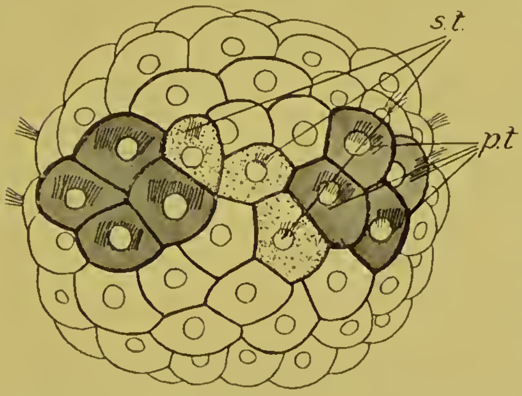

Fig. 232.—Lateral view of a late embryo of *Patella coerulea* showing the mode of completion of the prototrochal girdle. (After Wilson, altered.)

p.t, primary trochoblasts (darkly shaded); *s.t*, secondary trochoblasts (dotted).

By this time twenty-four hours have elapsed, and the embryo has escaped from the egg-membrane and entered on its free-swimming life as a Trochophore larva. In this larva the blastopore becomes shifted forwards and then completely closed, but the **stomodaeum** arises as an invagination of the ectoderm at the spot where the last trace of the blastopore disappeared. The shifting is due to the greater relative growth of the dorsal ectoderm behind the prototroch (Fig. 233). The endodermic cells divide and form a sac which is the **stomach**; and from the hinder end of this the **intestine**

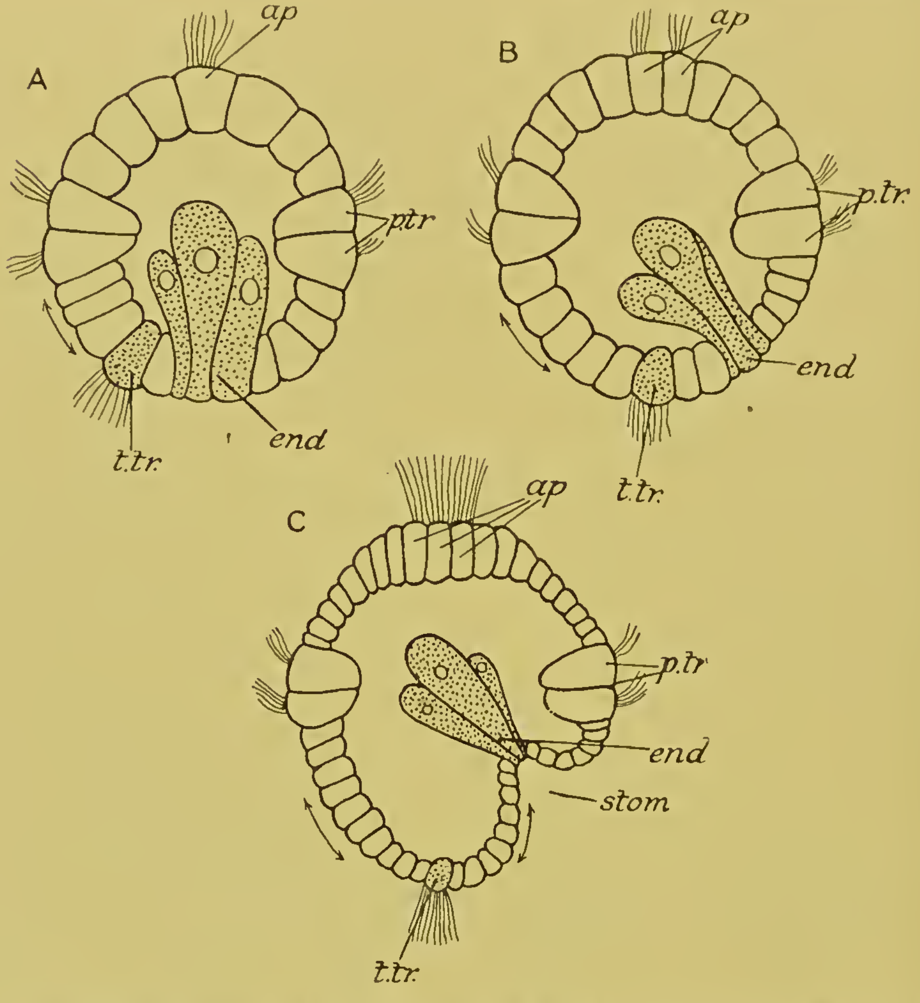

Fig. 233.—Three diagrams of sagittal sections of the early larva of *Patella coerulea* in order to illustrate the shift of the blastopore and the formation of the stomodaeum. (After Patten.)

A, stage where the blastopore is at the vegetative pole of the egg. B, stage where both telotroch and blastopore have been shifted forward owing to the growth of the dorsal region. C, stage where blastopore is shifted farther forward by growth of ventral region, the telotroch remaining stationary, and in which the stomodaeum has originated. The arrow indicates the regions of the embryo which are undergoing rapid growth. *ap*, apical plate; *end*, endodermal cells; *p.tr*, prototroch; *stom*, stomodaeum; *t.tr*, telotroch.

grows out as a narrow tube. The latter comes into contact with the ectoderm just behind a slight ventral projection carrying stiff hairs, which now becomes apparent and which corresponds to the **telotroch** of the larva of *Polygordius*: there the **anus** is formed later. There is, in addition to the telotroch, a mid-ventral band of very fine cilia passing forwards from the telotroch to the mouth, it corresponds to the mid-ventral ciliated groove found in many Annelid larvae, although it is not found in the larva of *Polygordius*.

There are, however, several points in which the larva has already passed the stage of the pure Trochophore larva and entered on a post-trochophoral stage of development. These are as follows: (1) In the dorsal region, behind the prototroch, a thickened plate of ectoderm cells has become recognizable, which is beginning to be slightly invaginated; this is the rudiment of the **shell gland,** the organ to which the shell owes its origin. (2) The mother cells of the adult mesoderm have proliferated, each giving rise to a short **mesodermal band.** (3) Two slight elevations at either side of the mid-ventral line have appeared, just behind the mouth, which are the beginnings of that characteristic Molluscan organ, the **foot.**

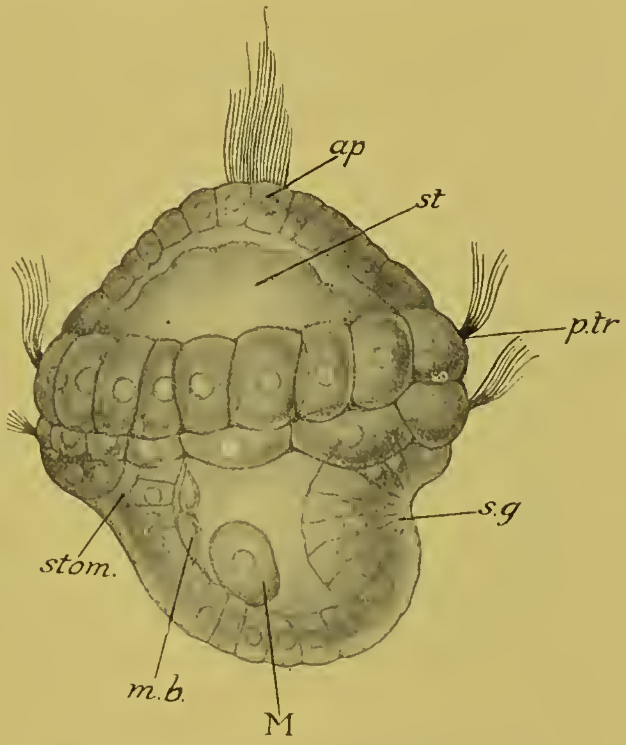

FIG. 234.—Lateral view of young Trochophore larva of *Patella coerulea*. (After Wilson, slightly altered.)

ap, apical plate; M, mother cell of mesoblast; *m.b*, mesodermal band; *s.g*, shell gland; *st*, stomach; *stom*, stomodaeum.

As development proceeds the life-history of the Mollusc diverges more and more widely from that of the Annelid. In the case of *Patella* the post-trochophoral stages of development have been described by Patten (1885); he was able to keep the larvae alive for a week, and has given a good account not only of the external appearance of the larva in these stages, but also, to some extent, of their structure, by means of sections.

Patten's account, although extremely interesting, is rather meagre, and whets the desire for a thorough reinvestigation; it is as follows. As the blastopore shifts forward and finally closes near the spot where the mouth will subsequently be formed, the two rudiments of the foot unite with one another in the mid-ventral line so as to form a median protuberance. The shell gland grows greatly in extent and depth so as to occupy the entire dorsal region behind the prototroch; the cells forming its floor become thin, whilst those constituting its sides remain thick, and subsequently the invagination shallows out and the floor becomes everted as a rounded hump. On this hump a thin thorny membrane is secreted, which is the first rudiment of the **shell** (Fig. 236). The eversion is due in large measure to the swelling up of the stomach. The thickened edge of the shell-gland region constitutes the rudiment of the **mantle fold**; under it appears a groove, deepest behind, and this deep spot is the rudiment of the **mantle cavity.** The rounded hump on which the cap-like shell is secreted is the **visceral hump.**

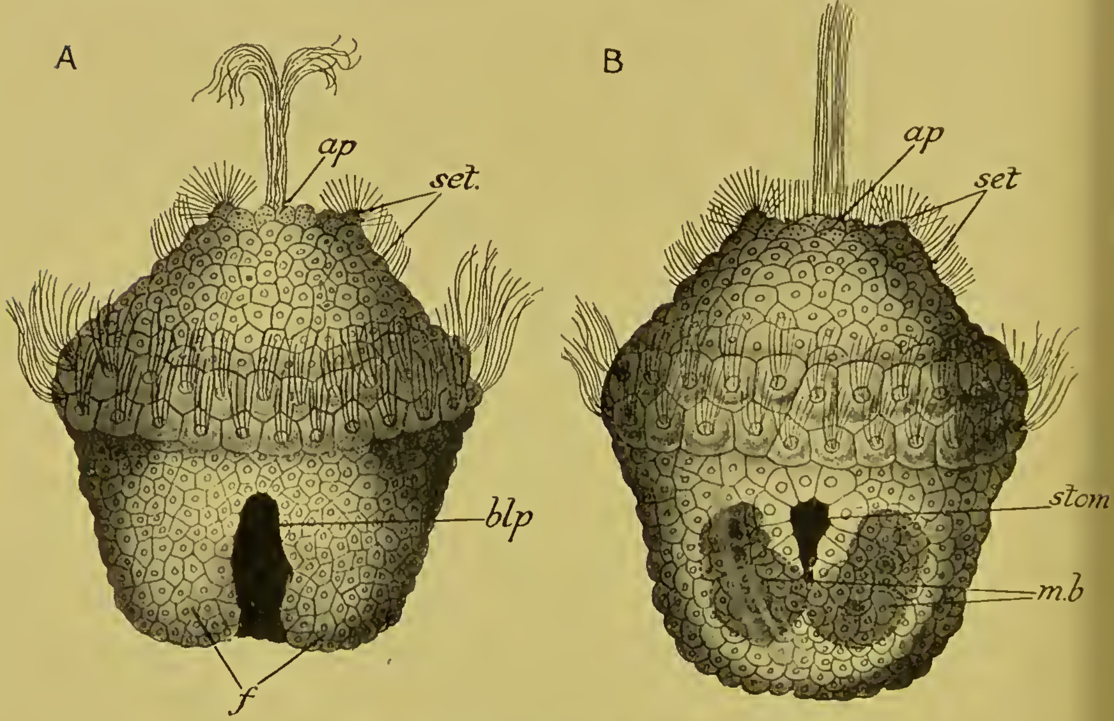

FIG. 235.—Ventral views of two stages in the development of the Trochophore larva of *Patella coerulea.* (After Patten.)

A, stage in which the blastopore extends to the posterior pole of the embryo. B, stage in which the blastopore is closed and the stomodaeum is formed. Letters as in the previous figure. In addition, *blp*, blastopore; *f*, foot; *set*, prominences in the pretrochal region bearing stiff setae.

The larva with its projecting foot and cap-like shell is termed a **veliger**; and its enlarged prototrochal girdle is called the **velum.** At first the visceral hump and shell project forwards, but in the latest stage observed by Patten they project backwards (Fig. 237). How this change was effected Patten did not observe; but it has been observed by Boutan (1899) in the closely allied genus *Acmaea*, and in other primitive Gastropoda, like *Fissurella* and *Haliotis,* which, like *Patella,* are members of the division Aspidobranchiata.

Patten made out some other interesting points in the development of *Patella.* The **radula sac** appears as a ventral pocket-like outgrowth of the stomodaeum (*r.s,* Fig. 236). The anterior ends of the mesodermic bands break up into loose tissue, like mesenchyme. Some of this tissue develops into long spindle-shaped cells, which are muscular and which are inserted at one end into the ectoderm of the visceral hump and at the other into the sides of the stomach.

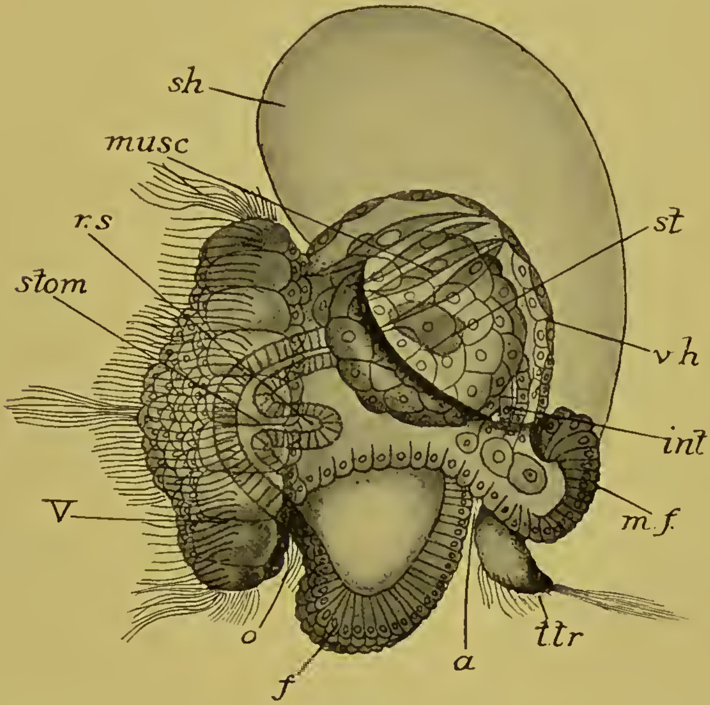

FIG. 236.—Side view of a veliger larva of *Patella coerulea* before torsion has taken place. (After Patten.)

a, position of anus; *f,* foot; *int,* intestine; *m.f,* mantle fold; *musc,* retractor muscle of the shell; *o,* mouth; *r.s,* radula sac; *sh,* shell; *st,* stomach; *stom,* stomodaeum; *ttr,* telotroch; V, velum; *v.h,* visceral hump.

Others of these cells form muscles connecting the apical plate with the sides of the oesophagus (as in *Polygordius*).

The **otocysts** are formed quite early, whilst the foot is still quite inconspicuous, as two elongated pits situated just behind the mouth on either side. Later, when the foot grows out and becomes very conspicuous, two ectodermic thickenings appear on its anterior surface, and are interpreted as the rudiments of the **pedal ganglia.**

What are most probably the rudiments of the **cerebral ganglia** are shown in Fig. 239, where, at the sides of the persistent apical organ with its powerful cilia, two ectodermic thickenings are seen.

The cerebral ganglia of *Patella,* on this supposition, would arise in the same way as those of *Polygordius,* at the sides of the apical plate (see Chap. VII).

The rudiments of the **eye cups** probably arise on the velar area at the sides of the apical plate, where two groups of three or four clear cells are seen, around which pigment is developed (*o.c,* Fig. 237). Curious prominences bearing stiff setae appear on the velar area at the sides of the apical plate. One is tempted to regard these as the rudiments of the tentacles, but according to Patten they disappear.

As the foot grows large the otocysts, which at first lie in front of it and which have become detached from the ectoderm, move into it. On the posterior aspect of the foot a thin **operculum** is developed, which is large enough to close the aperture of the shell. The shell itself, which was at first thin and chitinous, becomes much thicker and calcareous, with a corrugated surface. At the latest period at which Patten observed the prototrochal girdle or velum, it consisted of three concentric rows of cells, a middle row of very large tall cells carrying very powerful cilia, and an anterior and posterior circle of cells carrying much smaller cilia.

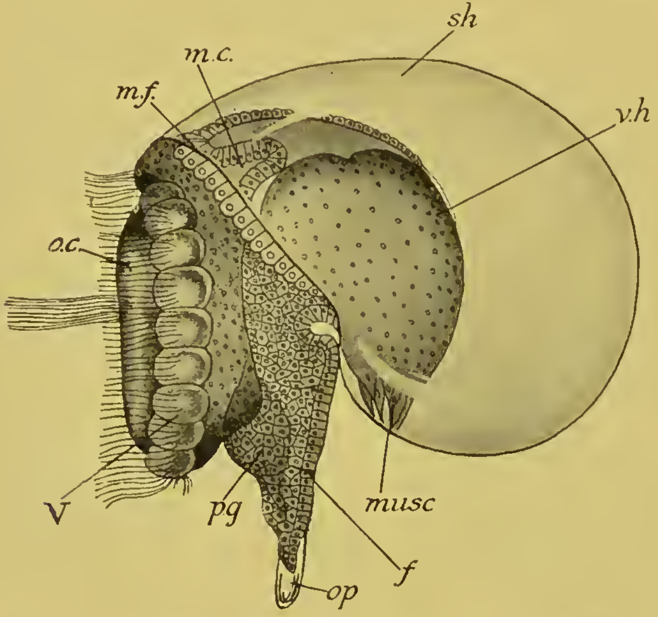

FIG. 237.—Side view of a veliger larva of *Patella coerulea* after torsion has taken place. (After Patten.)

Letters as in previous figure. In addition, *m.c,* mantle cavity ; *o.c,* rudiment of eye ; *op,* operculum ; *p.g,* rudiment of pedal ganglion.

It will be seen that a thorough investigation of the manner in which the organs of *Patella* are built up has yet to be made; Patten's description must be regarded as a first sketch. He did not succeed in keeping his larvae alive for more than a week. Recently, however, the larvae of *Patella vulgata* have been reared through their

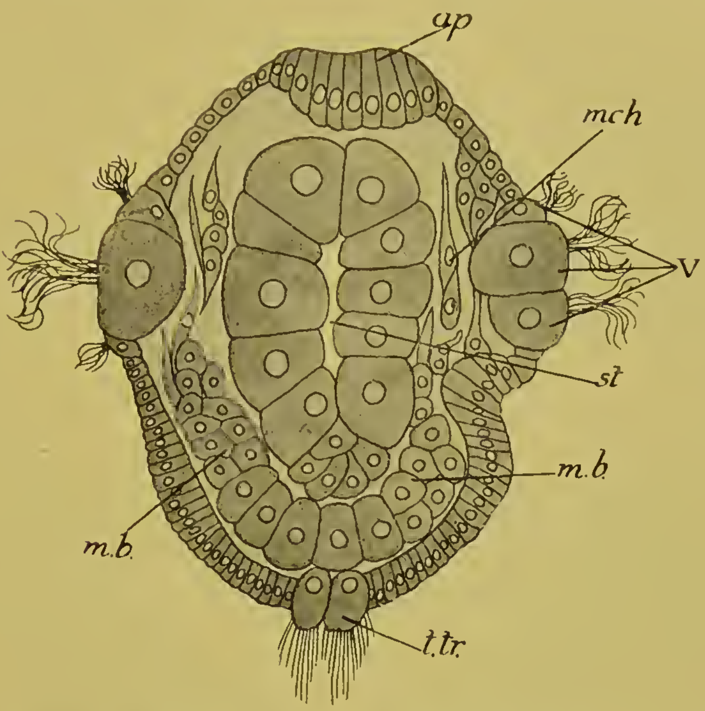

FIG. 238.—Frontal section of a veliger larva of *Patella coerulea* in order to show the mesodermic bands. (After Patten.)

ap, apical plate; *m.b*, mesodermic band; *m.ch*, mesenchyme cells budded off from the front ends of the mesodermic bands; *st*, stomach; *t.tr*, telotroch; V, velum.

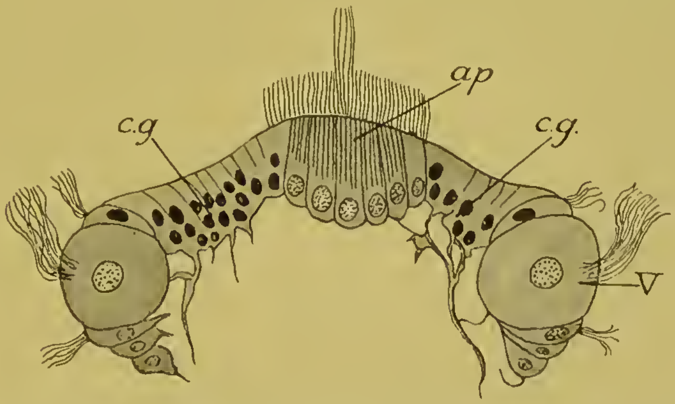

FIG. 239.—Frontal section through the pretrochal region of an old veliger larva of *Patella coerulea*. (After Patten.)

Letters as in previous figure. *c.g*, rudiment of cerebral ganglion.

entire metamorphosis, until they assume the adult form. This result has been accomplished in Plymouth, using *Nitschia* as food. The field is therefore open for a renewal and revision of Patten's work.

OTHER GASTROPODA

It has been mentioned above that the metamorphosis into the adult form, so far as its external features are concerned, has been worked out in *Acmaea*, *Fissurella*, and *Haliotis* by Boutan (1899). Taking *Acmaea* as an example, since it is closely allied to *Patella*, we find, according to Boutan, that as the visceral hump becomes longer, the alimentary canal, consisting of larval stomach and intestine, becomes bent into a U shape; the mouth and anus being comparatively near each other, separated only by the small foot. Suddenly the incipient mantle-cavity, and the anus which opens into it, which were originally situated in the middle line behind, become twisted upwards and forwards so as to open on the neck of the larva. According to Boutan this **torsion**, which is due to the unequal growth of the two sides of the larva, takes place with great rapidity, and as a result of it the apex of the visceral hump hangs backwards (cf. Figs. 237 and 238).

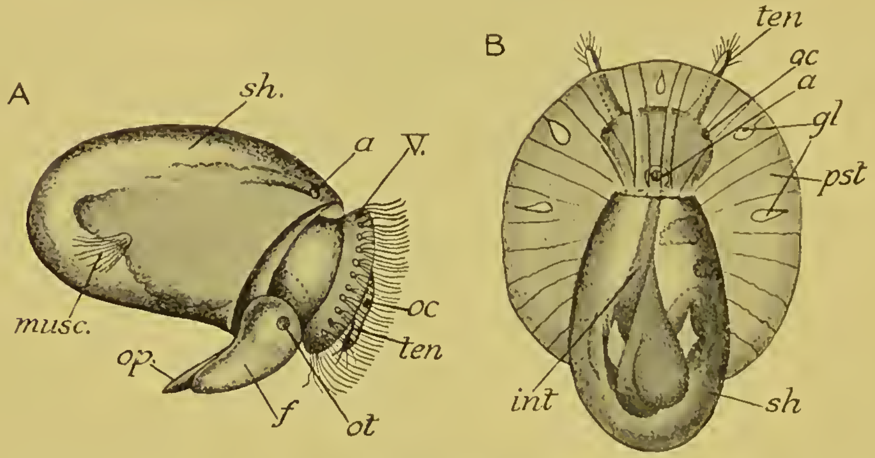

Fig. 240.—Two views of the advanced stages of development of *Acmaea virginea*. (After Boutan.)

A, Lateral view of veliger larva. B, Dorsal view of young *Acmaea* just after the velum has been cast off. *a*, anus; *f*, foot; *gl*, glands in the mantle roof; *int*, intestine; *musc*, retractor muscle of shell; *oc*, eye; *op*, operculum; *ot*, otocyst; *pst*, peristome; *sh*, larval shell; *ten*, tentacle; V, velum.

The growth of the mantle edge continues to be uniform all round its periphery, and produces an everted, conical lip to the shell, which is termed the **peristome.** At the sides of the apical plate the eyes and tentacles have made their appearance. Soon the velum is cast off and the young mollusc sinks to the bottom. The peristome continues to grow until it forms the adult conical shell; finally the visceral hump is withdrawn from the tiny cap-like larval shell and it is cast off, and thus the adult state is attained. There is no reasonable doubt that the later development of *Patella* is in all respects similar to that of *Acmaea*.

In *Fissurella* the development is essentially similar; in this genus also there is a cap-like larval shell which is eventually cast off; but in the expanded peristome a notch appears, due to a corresponding indentation of the mantle edge. As growth continues this notch is pushed further and further up towards the apex of the adult shell, because the indentation in the mantle edge becomes converted into a hole by the formation of a bridge of tissue across its lower end; the mantle edge in this way reacquires a smooth, rounded margin, and then secretes, in later periods of growth, concentric unbroken rings of peristome.

In *Haliotis* the larval shell persists throughout life and the notch in the mantle edge is permanent, and as a result a row of holes in the adult shell is produced, since partial unions of its edges, which secrete bridges of shell, are formed across its upper and older part. In *Haliotis* the growth of the peristome is not quite even on the two sides of the mantle edge, and as a consequence the embryonic shell becomes pushed to one side. This is the first indication of the spiral twist so conspicuous in the shells of most Gastropoda.

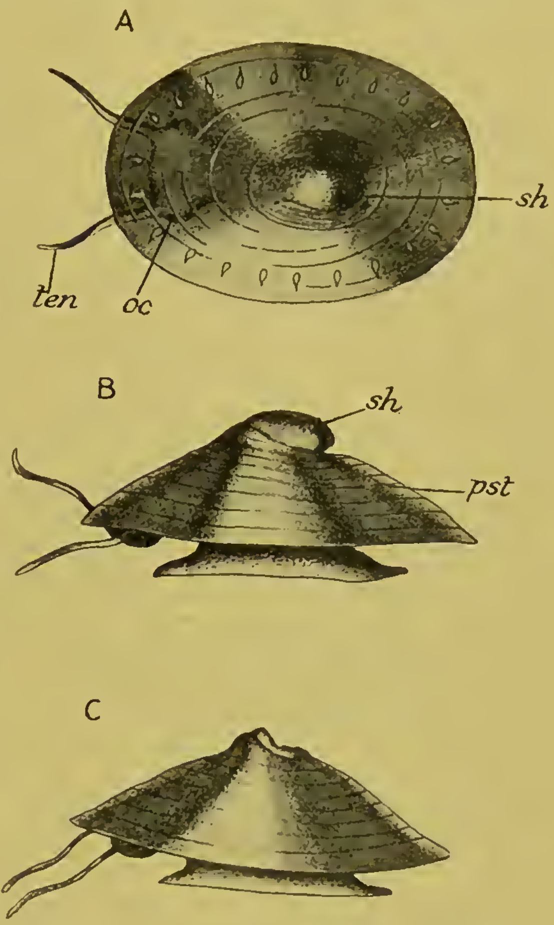

FIG. 241.—Three views of the just-metamorphosed *Acmaea virginea* to show the formation of the adult shell and the loss of the larval shell. (After Boutan.)

A, dorsal view of stage in which larval shell is retained as an apical knob. B, lateral view of a similar stage. C, lateral view of stage in which the larval shell has been cast off. Letters as in previous figure.

EXPERIMENTAL EMBRYOLOGY OF PATELLA

We now pause to consider a question which must have presented itself to the mind of the reader when we described in detail the typical seriation of cell divisions in *Polygordius*: namely, are these cell divisions important as separating materials destined to form various organs, or are they merely a means for effecting an approximately even distribution of nuclear material?

No experiments have been made which will allow us to answer this question in the case of *Polygordius*, but in the case of the essentially similar development of *Patella* the question has been answered by Wilson. Herbst has shown that if the segmenting eggs of marine Invertebrates are deprived of their fertilization membranes by shaking, and are then exposed to the influence of an artificial sea-water from which calcium has been excluded, the blastomeres which are formed by successive cleavages fail to cohere, and instead of a Metazoon consisting of many cells, a heap of isolated cells is produced.

This method was applied by Wilson (1904) to the study of *Patella*, as follows. The eggs were allowed to segment in normal sea-water until they had attained the 16, 32, or 64-cell stages. They were then transferred to artificial sea-water devoid of calcium, and when the blastomeres had separated from one another either completely or so as to form small groups of cells, the isolated blastomeres or groups of blastomeres were returned to normal sea-water and allowed to develop further. The tendency to spontaneous isolation does not, however, immediately cease when this is done, and so, for the earliest stages, Wilson did not use artificial sea-water to produce separation, but divided the first two blastomeres from one another by means of a fine scalpel under a dissecting microscope. Even for later stages he found it advisable not to wait till the separation had been completely effected by the artificial sea-water, but as soon as loosening had taken place he completed the separation either by a scalpel or by blowing a jet of water on the egg through a fine pipette. When the experiment was performed on a larva which had begun its free-swimming life, the method adopted was simply to leave it in the artificial sea-water for twenty-four hours, without

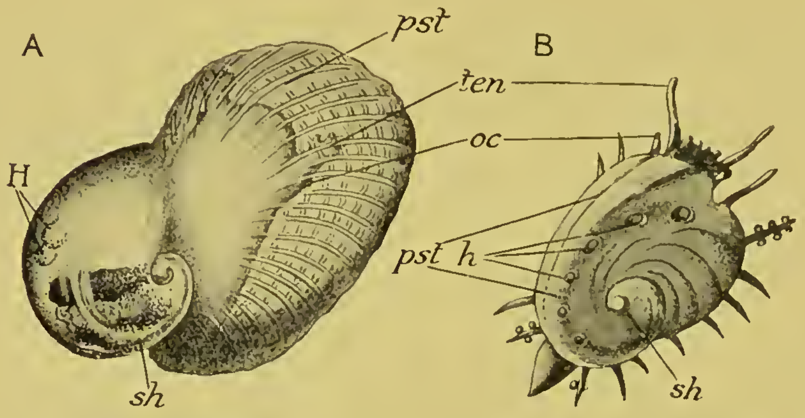

FIG. 242.—Two views of the young *Haliotis tuberculata* to show the formation of the adult shell. (After Boutan.)

A, stage in which the larval shell is still relatively large. B, stage in which the larval shell is relatively very small, and in which the holes in the adult shell have appeared. [B is less magnified than A.] Letters as in two preceding figures. In addition, H, heart; *h*, holes in adult shell.

aiding the effect of the water either by shaking or cutting the larva with a scalpel.

An isolated blastomere of the 2 and 4-cell stages was found to be exceedingly difficult to rear; it always segmented as if it formed part of a complete embryo. In most cases the resulting mass of cells flew into pieces without producing anything which could be called a larva. In a few cases, however, this disruption did not happen, and then there resulted a larva of diminished size and of the **closed** or **open** type. Such larvae may be termed **closed,** or **open dwarf larvae** respectively. By the latter term is meant a larva in which the half or quarter ectoderm does not cover the whole egg, by the first term a larva in which, by secondary shiftings of the cells, the macromeres are entirely covered by ectoderm. In the closed type of larva, in spite of these secondary shiftings, each cell undergoes the fate which would have befallen it had it formed part of a perfect egg; thus a larva developed from a single blastomere of the 2-cell stage produces only two groups of primary trochoblasts, and a larva from a blastomere of the 4-cell stage only one. It is obvious that in these closed larvae, each cell forms a larger part of the periphery of a sphere than it would normally do; and it must, therefore, be subjected to a considerable strain in order to produce such an abnormally sharp curvature, and this strain may account for the explosive character of these dwarf larvae. These larvae, if they live, always finally "close," and then gastrulation occurs: they always produce an apical organ.

Much greater success attended Wilson's efforts to separate the micromeres of the 8-cell stage. These when isolated segmented exactly as if they still formed part of the whole egg; at the end of twenty-four hours they were converted into little ectodermic vesicles, with an apical organ at one end and a group of four primary trochoblasts with their long powerful cilia at the other (Fig. 243, A and B). When the entire group of the first four micromeres were isolated they also segmented as if they still formed part of the entire egg; but they proved to be a very unstable combination, some cells always separated, and the largest dwarf larva that was obtained represented the products of the division of only three micromeres.

When the products of the division of the first quartette of micromeres were isolated, similar results were obtained. Such cells always segmented as if they formed part of the entire egg, and later endeavoured to round themselves off and form ectodermic vesicles. Thus, cells belonging to the group $1q^1$ developed into ectodermic vesicles with an apical organ at one end and two secondary trochoblasts at the other; these secondary trochoblasts being at once distinguishable from the primary ones by the smaller size of their cilia. Cells belonging to the group $1q^2$ divided into a group of four primary trochoblasts; cells belonging to the groups $1q^{21}$ or $1q^{22}$ divided once and produced a pair of trochoblasts; cells of any of the groups $1q^{211}$, $1q^{212}$, $1q^{221}$, or $1q^{222}$, when isolated, did not divide, but acquired the long powerful cilia characteristic of a trochoblast.

The macromeres of the 8-cell stage, *i.e.* 1A, 1B, 1C, and 1D, when isolated, usually either died or formed masses of cells which disintegrated; but in some few cases larvae were obtained. In all cases a second quartette cell was formed, which divided as it normally does, into a lozenge-shaped group of four cells; then a third quartette cell was formed which divided into two, and a cell belonging to the fourth quartette was also produced.

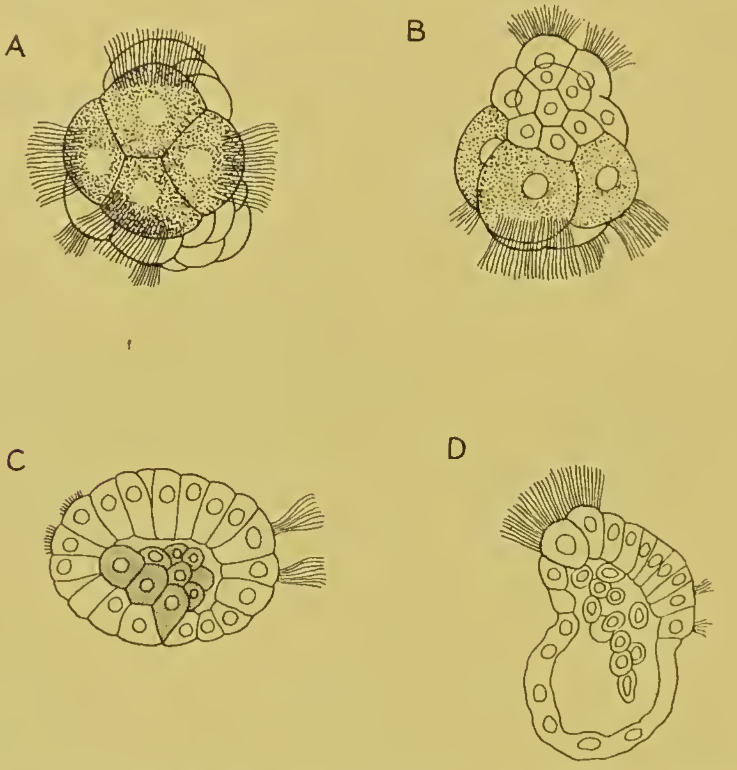

FIG. 243.—Illustrating the results obtained by separating the blastomeres of the developing egg of *Patella coerulea.* (After Wilson.)

A, under view. B, lateral view of the larva, resulting from the development of a single isolated micromere of the first quartette. The four primary trochoblasts are shaded. The other cells bearing cilia in B are apical cells. C, lateral view of the larva, resulting from the development of one of the four macromeres of the 8-cell stage. Two secondary trochoblasts are seen, and two feebly ciliated cells, which are probably a portion of the mid-ventral ciliated groove. The endoderm cells are shaded. D, lateral view of the larva, resulting from the development of one of the micromeres of the second quartette. Two secondary trochoblasts are seen, and two feebly ciliated cells, probably a portion of the mid-ventral ciliated groove. The interior of the larva contains loose cells representing the so-called mesectoderm.

When a larva was obtained from one of these isolated macromeres the mass of cells belonging to the second and third quartettes were seen to have undergone such displacement as to form a complete ectodermic covering for the macromeres, and gastrulation had occurred. At the one end of the embryo were two cells bearing powerful cilia. These were obviously the secondary trochoblasts originating from the second quartette; and the fact that there were two, suggests that normally two cells are contributed to the formation of the prototroch in each quadrant by this quartette. At the other end of the larva were two feebly ciliated cells. These appear to be cells belonging to the third quartette which normally form part of the **ventral ciliated groove** of the larva, they bear exceedingly fine cilia (Fig. 243, C).

If one of the residual macromeres 2A, 2B, 2C, or 2D was isolated, *i.e.* a macromere of the 16-cell stage, a very similar development was obtained. Of course, only cells of the third and fourth quartette were produced, and the formation of a complete larva was very rare. Nevertheless in a few cases this did occur. When it did occur the larva had an internal endodermic mass of cells; but it had no trochoblasts, though two of its cells bore weak cilia. These latter would have formed part of the ventral ciliated band if they had been a portion of a whole larva.

When micromeres of the second quartette were isolated they produced, like the micromeres of the first quartette, ovoid ectodermic vesicles with one or two ciliated cells at one end. These are the one or two secondary trochoblasts that are normally produced from this quartette. The vesicles often have some cells in the interior, and these cells are almost certainly the so-called **mesectoderm** or **larval mesoderm**, *i.e.* cells which sink into the blastocoele and normally produce larval muscles. Such cells have been described in *Polygordius* and in the development of other Mollusca, and they will certainly be found to exist in the normal development of *Patella* when this has been exhaustively analysed (Fig. 243, D).

Reviewing the experiments which have been described, we see that they prove conclusively that cells from the early stages of *Patella*, when isolated, give rise to nothing different from what they would have given birth to had they remained part of the egg, and that therefore the cleavage of the egg, from the first, separates definite **organ-forming substances**. We are dealing in fact with an egg with specialized structure like the Ctenophore egg, and one which is more specialized in this respect than the Nemertine egg, since the product of one of the first two, or one of the first four blastomeres, is not a dwarf larva of half or quarter size, but a monstrous being with only one-half or one-quarter (as the case may be) of the larval structures. Thus, our views as to the affinities of Mollusca with the Ctenophora, which we deduced from the appearance of the early larva of *Patella*, are strengthened by the constitution of the egg as revealed by these experiments.

PALUDINA

It has been mentioned above that the formation of the internal organs has not been fully worked out in *Patella*; nor has it been done satisfactorily in the case of any Mollusc except *Paludina*.

Paludina is, like *Patella*, a univalve or Gastropod, but it differs from *Patella* in possessing a spirally coiled shell. It is a fresh-water form found abundantly on both sides of the Atlantic. Its peculiarity is that the lower part of the oviduct is enlarged to form a kind of womb within which the eggs undergo their complete development, leaving the body of the mother only when they have attained, externally at least, a complete likeness to the adult. If a number of adults, then, be collected and killed in an extended condition, by the slow addition of minute quantities of chloral or cocaine to the water in which they are living, and if the shells be then carefully picked away piece by piece and the oviducts slit open, the contained embryos may be washed out by aid of a pipette into a watch glass of normal salt solution, examined fresh, and afterwards preserved by the corrosive sublimate and acetic acid mixture.

The egg is very minute; it divides regularly into blastomeres of nearly equal size, the micromeres being as big as the macromeres; it forms a regular trochophore and then a veliger; in fact it pursues a primitive development within the oviduct, by the secretion of which it is nourished, and it does not depend for sustenance on the yolk contained in its own cells.

We owe to Erlanger (1891, 1892) an exhaustive account of the development of *Paludina*; and his results, so far as the later stages are concerned, have been confirmed and extended by Miss Drummond (1902). So far as the earliest stages are concerned, however, Tönniges (1896) has directly contradicted Erlanger's statements; and on this controversy a few general remarks may be made.

Erlanger's account, written before the days when cell-lineage was studied, commences with the gastrula stage. This stage is reached by a process of regular invagination such as is found in *Polygordius*, not by a massive inflow of large cells as in *Patella*. The blastopore, according to him, persists as the **anus**, and the **stomodaeum** is formed in front of it, so that the mouth is a new perforation. The prototroch appears, as in *Patella*, as a double circle of cilia carried by two rows of cells. *On the ventral side of the intestine a median bilobed pouch is formed, which is the origin of the mesoderm* (Fig. 245). This pouch becomes cut off from the gut, loses its cavity, and gives rise to two irregular mesodermal bands which extend forwards at the sides of the gut. Each of these bands gives off a small compact mass at its anterior end (*l.n*, Fig. 246), which becomes converted into a **larval kidney** (Erlanger, 1894); while the rest of the streak breaks up into an irregular mass of stellate cells

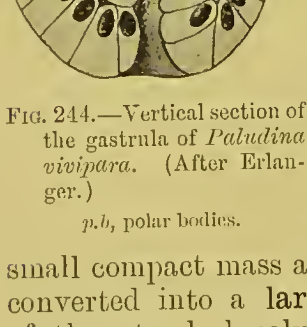

Fig. 244.—Vertical section of the gastrula of *Paludina vivipara*. (After Erlanger.)

p.b, polar bodies.

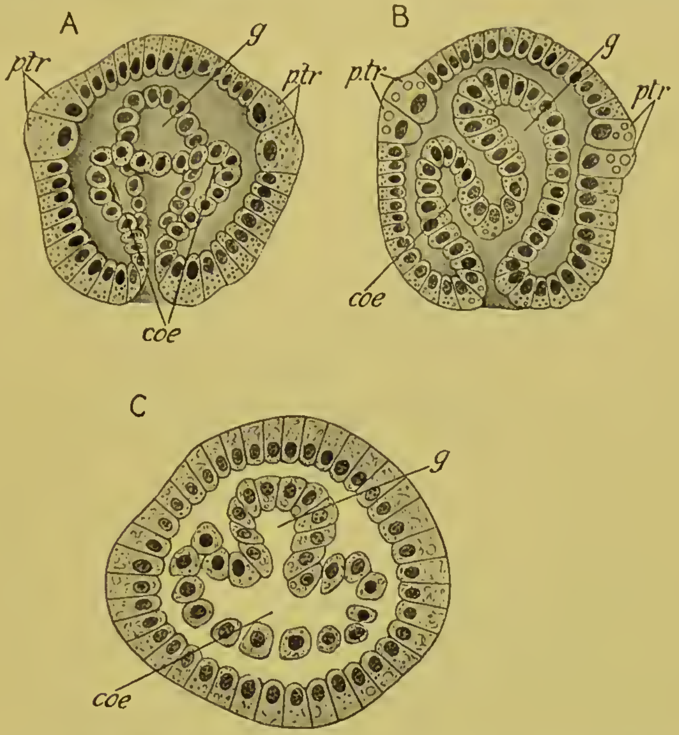

FIG. 245.—Formation of the coelom in *Paludina vivipara*. (After Erlanger.)

A, optical frontal section of embryo in the stage when the coelom is being formed. B, sagittal section of embryo in this stage. C, transverse section in this stage. *coe*, coelomic pouch; *g*, gut; *p.tr*, prototrochal cells.

which span the **blastocoele**, extending from gut to ectoderm. In the hinder end of each streak there is, however, a compact mass which becomes hollowed out to form a **coelomic vesicle**, the rudiment of one of the **pericardial** sacs. The two mesodermic bands then fuse together in the middle line behind, and the pericardial sacs become pressed against one another so that their conjoined walls form a septum (*sept*, Fig. 247, B).

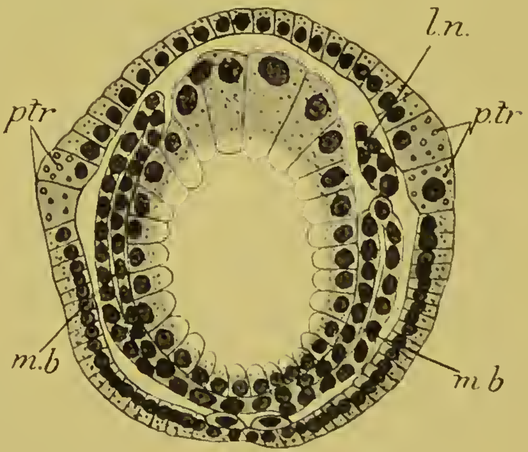

FIG. 246.—Optical frontal section of an embryo of *Paludina vivipara* a little older than those represented in figure 245. (After Erlanger.)

l.n, larval kidney; *m.b*, mesoblastic band; *p.tr*, prototrochal cells.

Tönniges denies point-blank the existence of this ventral sac. According to him the adult mesoderm arises as an ectodermal proliferation, on each side of the middle line, which gives rise to the irregular mass of cells seen by Erlanger. Now, we may quite confidently say that, whatever may be the true state of affairs, Tönniges is most certainly wrong. For what he figures as the earliest stages of the formation of the mesoderm in *Paludina* are precisely similar to later stages in the development of the mesoderm in *Physa*, and other forms, whose cell-lineage has been worked out in the greatest detail. In all these cases the origin of the adult mesoderm has been traced to the cells of the fourth quartette, which, as in Annelida, are part of the endoderm.

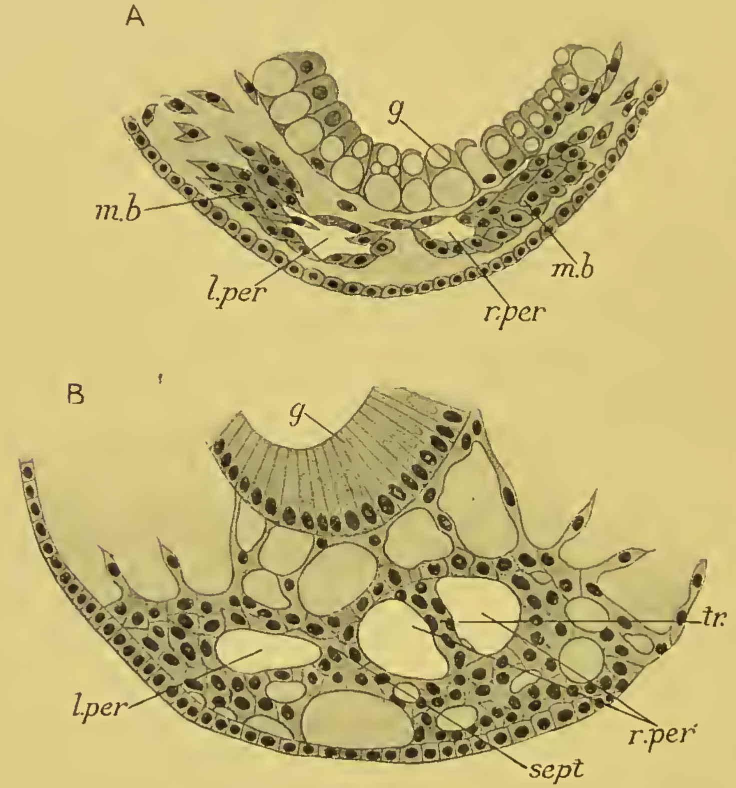

FIG. 247.—Two stages in the formation of the pericardium of *Paludina vivipara.* (After Erlanger.)

A, horizontal section through visceral hump, of stage in which the two mesodermic bands are still separate—a rudiment of the pericardium has appeared in each. B, horizontal section through visceral hump of later stage, in which the two mesodermic bands have fused in the middle line to form the septum separating the right and left pericardial sacs. *g*, gut; *l.per*, left pericardial sac; *r.per*, right pericardial sacs; *sept*, septum formed by the opposed walls of the pericardial sacs; *tr*, trabecula of cells crossing right pericardial sacs.

The later products of the division of the mother cells of the mesoderm, it is true, often come into such close contact with the ectoderm that, if one had not a complete series of the earlier stages to examine, one would believe that there was demonstrative proof that the mesoderm was derived from the ectoderm; and indeed this very mistake has been made by other German workers in the case of other Mollusca (Meisenheimer, 1898, 1901, and Harms, 1909). The difficulty in the case of *Paludina* is that the complete series of earlier stages is not at all easy to obtain, since the earlier stages of development are passed through rapidly while the later stages of growth take much longer to accomplish. The chances are, therefore, that in any one womb nearly all the embryos will belong to the post-trochophoral or veliger stages; and Erlanger himself once told us that to find material for the adequate study of these earlier stages would require at least two months' search. The opportunities for coming to a decision in the matter are far fewer than in the case of an ordinary Gastropod, whose eggs are laid by thousands, and where every desired stage can be had in abundance.

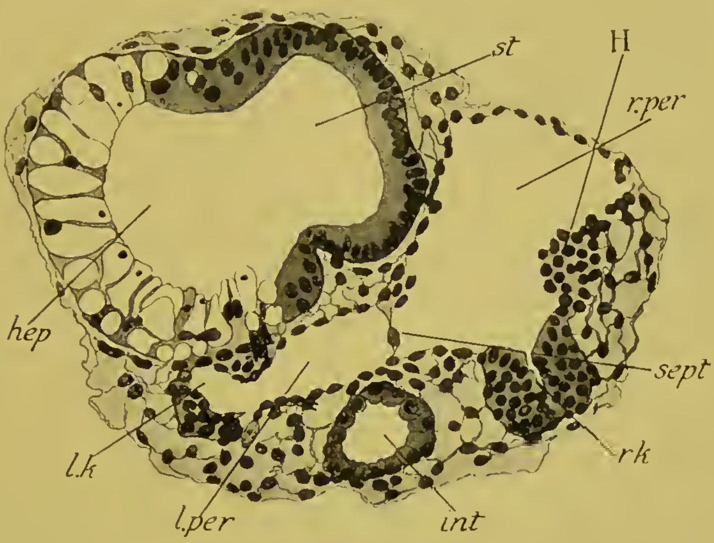

FIG. 248.—Horizontal section through the visceral hump of an older embryo of *Paludina vivipara* than that represented in Fig. 247, to show the formation of the kidneys and the heart. (After Drummond.)

H, rudiment of heart; *hep*, liver; *int*, intestine; *l.k*, left kidney; *l.per*, left pericardial sac; *r.k*, right kidney; *r.per*, right pericardial sac; *sept*, evanescent septum between the pericardial sacs; *st*, stomach.

Erlanger's account of the matter agrees in *principle* with what is known of the development of Annelida with very small eggs, like *Eupomatus* (*Hydroïdes*), where the mesodermal cells are budded out from the intestine. But of course the formation of a definite pouch is a far more primitive method of development than that so far described for any Annelid or Mollusc, and it is a somewhat strange thing that this mode of development should be found in *Paludina*, which cannot be described as a very primitive member of the class to which it belongs.

There the matter must rest, since Erlanger has been cut off by an untimely death, till some other embryologist has the patience to thoroughly investigate this difficult subject.

When we reach the stage of the development of the pericardium of *Paludina*, agreement happily reigns among observers. The **foot** appears as a mid-ventral protrusion, the **shell gland** as a mid-dorsal shallow invagination. Just as in the case of *Patella*, the shell gland is everted and converted into a shell-forming area covering a **visceral hump**. The **mantle fold** and **mantle groove** appear in the same way as in *Patella*, and the torsion process takes place apparently slowly, as all stages in its completion are often found.

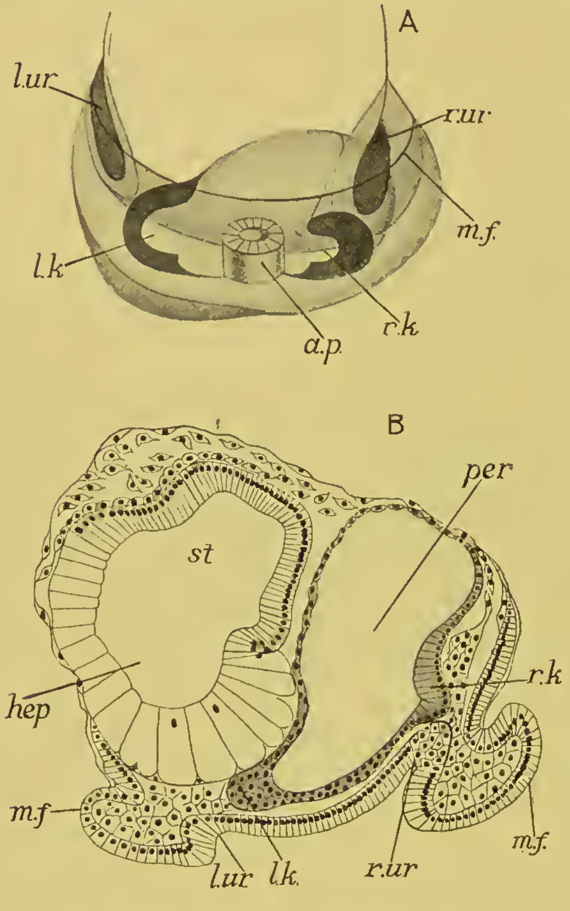

FIG. 249.—Illustrating the development of the ureters of *Paludina vivipara* and their relation to the kidneys. (After Erlanger.)

A, cut-off visceral hump of an embryo, rather older than that represented in Fig. 248, viewed from below. B, horizontal section through the visceral hump of an embryo of the same age as that represented in A. *a.p*, anal papilla; *hep*, liver; *l.k*, left kidney; *l.ur*, left ureter; *m.f*, mantle fold; *per*, pericardial sac (the right and left pericardial sacs of the earlier stage have fused); *r.k*, right kidney; *r.ur*, right ureter.

Before this happens, however, the rudiments of many organs appear. To begin with, the compact mass at the hinder end of each mesodermic band becomes hollowed out, as we have already seen, to form a small pericardial vesicle (Fig. 247), and from each of these vesicles an evagination, the rudiment of a **kidney**, is found. The two coelomic vesicles are at first separated by a septum, but this is soon absorbed and a single vesicle, the **pericardium**, results (Fig. 249). This lies ventral to the intestine near the posterior end of the embryo. The rudiment of the **heart** appears as a dip in the dorsal wall of this sac, and in this way a bag full of blastocoelic fluid is formed which hangs down into the pericardium and constitutes the heart with its contained blood.

The kidney on the right side becomes marked off from the pericardium by a constriction, and this narrow communication forms the **reno-pericardial canal** of the adult. On the left side the kidney rudiment remains small and thick-walled, and is also marked off from the pericardium by a constriction.

The **ureters** or external sections of the kidneys arise as ectodermal invaginations. They are in reality, however, only deeper portions of the mantle groove which intervenes between the mantle fold and the body wall (Fig. 249).

The embryonic stomach, whose cells are gorged with albuminous matter, has been shifted into the visceral hump, and in this way the alimentary canal takes on a U shape. The intestine is lined by small cubical cells, and, by an extension of cells of this description along the mid-dorsal and mid-ventral lines to meet the stomodeal cells, the embryonic stomach becomes divided into two lobes which, in later life, become converted into the two lobes of the **liver.** The median portion lined by small cells forms the adult stomach, and the **radula sac** arises as a ventral pocket of the stomodaeum.

Meanwhile the rudiments of the sense organs appear. The **otocysts** (*ot*, Fig. 250) arise as pocket-shaped invaginations of the ectoderm at the side of the foot. The **eyes** arise as similar invaginations of the pretrochal area (*oc*, Fig. 250); they are formed at the bases of two conical projections which are rudiments of the **tentacles** (*ten*, Fig. 250).

The principal ganglia of the central nervous system arise as separate ectodermic thickenings, the commissures connecting them being formed only afterwards. The **cerebral ganglia** arise later as two thickenings of the velar region close to the eyes. The **pedal ganglia** arise similarly from the post-velar region close to the otocysts, at the sides of the foot. The **pleural ganglia** arise on the sides of the body higher up and further back; and lastly the **visceral ganglia** arise from the ectoderm of the mantle cavity, that is, the deepest and most posterior part of the mantle groove. All these ganglia and their commissures are established before torsion begins.

Torsion now takes place, and the mantle cavity, with the opening of the anus and the two visceral ganglia, is rotated upwards and to the right, so that it passes along the right side in an oblique line until it reaches its permanent position on the back of the neck. This torsion involves the lengthening of the intestine into a recurrent loop, and the passage of the original right visceral ganglion upwards and to the left, where it forms the **supra-intestinal ganglion,** whilst the original left one is displaced to the right side and forms the **sub-intestinal ganglion.** The right ureter passes upwards and to the left, and the left one eventually takes up a position on the right below it (Fig. 250, B). The pericardium and the persistent right kidney are displaced from their original position, underneath the gut, to a lateral position in which the kidney is above the pericardium.

When the torsion is complete the velar cells disappear and the tentacles become long, while the foot develops its crawling surface, and on the upper aspect of its posterior portion the operculum appears. The **gill** appears as a series of outgrowths from the roof of the mantle cavity, and the embryo then takes on the general appearance of the adult (Fig. 251).

After the embryo has escaped from the womb of the mother, the **genital organs** develop. According to Miss Drummond (1902), the genital cells are budded from the pericardial wall, close to where the original left kidney joins it, and this kidney forms the first part of the **genital duct.** Coincidently with this development the pericardium is shut off from the genital rudiment. The outer and longer portion of the genital duct is formed by the left ureter (Fig. 252).

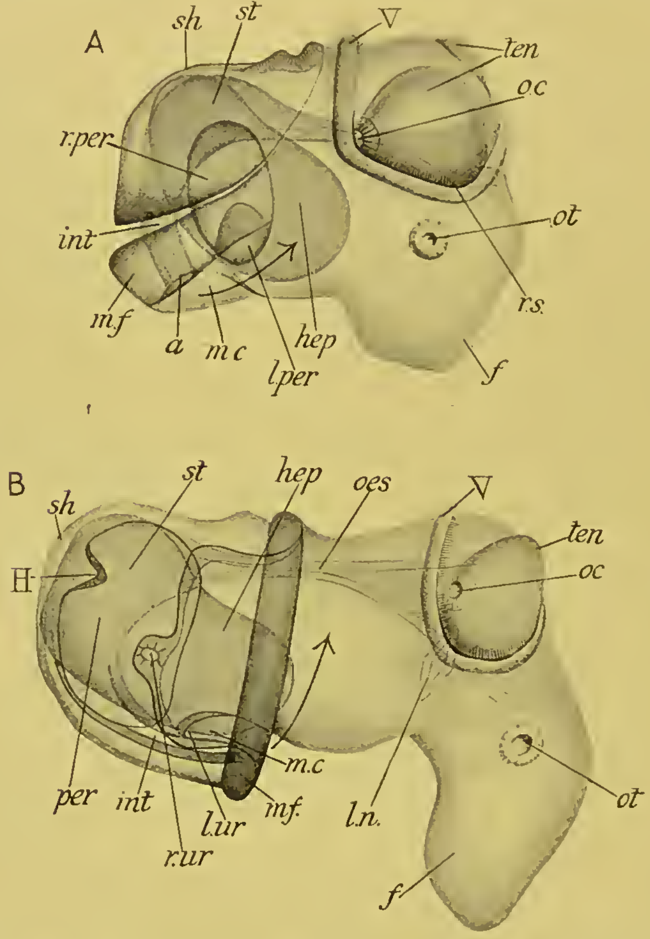

Fig. 250.—Two embryos of *Paludina vivipara* viewed from the right side in order to show the origin of the sense-organs and the beginning of torsion. (After Erlanger.)

A, stage when the foot and visceral hump are both short, and the embryo is almost bilaterally symmetrical. B, stage when foot and visceral hump have become elongated, and in which torsion has begun. The arrow shows the direction in which torsion takes place. *a*, anus; *f*, foot; H, heart; *hep*, liver; *int*, intestine; *l.n*, larval kidney; *l.per*, left pericardial sac; *l.ur*, left ureter; *m.c*, mantle cavity; *m.f*, mantle fold; *oc*, eye; *oes*, oesophagus; *ot*, otocyst; *r.per*, right pericardial sac; *r.s*, radula sac; *r.ur*, right ureter; *sh*, shell; *st*, stomach; *ten*, tentacles; V, velum.

The **torsion** of the visceral hump, which results in the transference of the mantle cavity from the posterior aspect of the hump to its anterior face, is to be carefully distinguished from the **spiral twisting** of the hump, which is shown by the spirally twisted shell; since the shell may never be spirally twisted at all, as in *Acmaea*, and presumably in *Patella*, and yet the torsion may reach its extreme limit; moreover, torsion is always complete before the spiral twisting of the shell begins, as is well seen in the development of *Paludina*. Both changes are due to the unequal growth of the two sides of the animal; but whereas torsion affects the whole area of the side of the body above the insertion of the foot, the inequality of growth resulting in spiral twisting of the shell affects only a more dorsal region, leaving the floor of the mantle cavity unaffected. It is accompanied by a lengthening of the visceral hump—and this is associated by Miss Drummond (1902) with the outgrowth of the embryonic stomach so as to form the adult liver. The most plausible explanation of the inequality of growth, in both torsion and twisting, is that in the ancestral gastropod the lengthened visceral hump fell over to one side as the animal took to crawling over uneven ground. As a

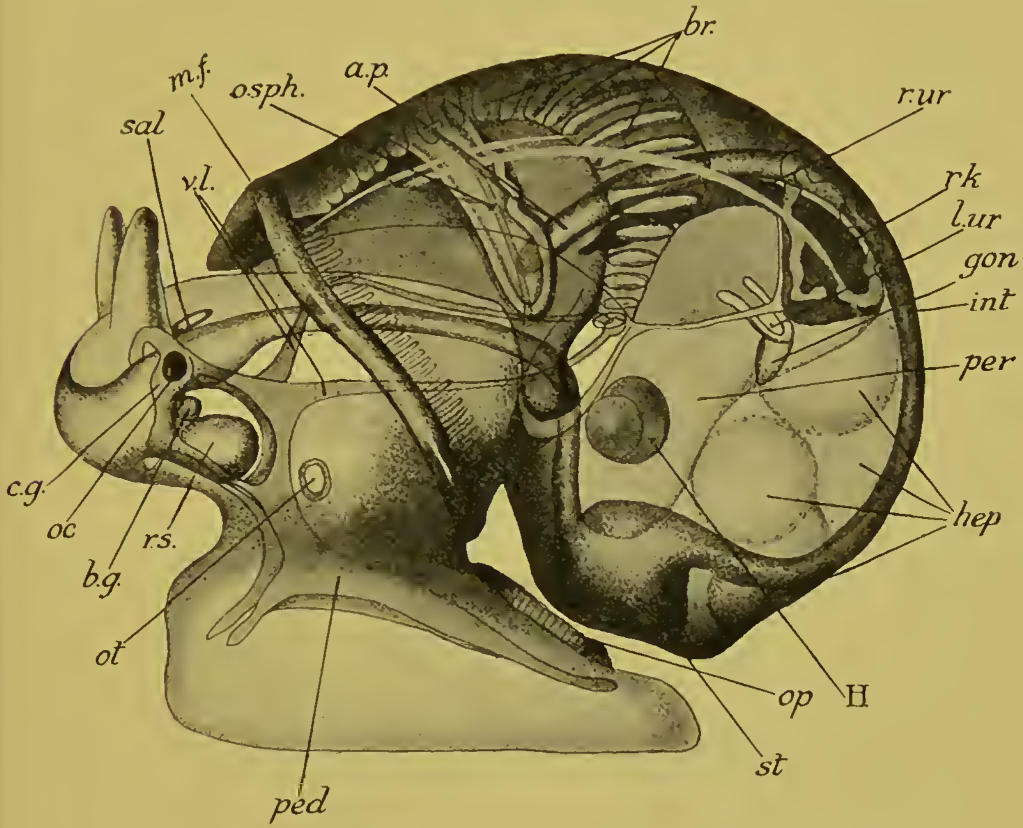

FIG. 251.—Just hatched *Paludina vivipara*. Seen from the left side and viewed as a transparent object. (After Erlanger.)

a.p, anal papilla; *b.g*, buccal ganglion; *br*, rudiments of the gill; *c.g*, cerebral ganglion; *gon*, rudiment of genital organ; H, heart; *hep*, liver; *int*, intestine; *l.ur*, left ureter; *m.f*, mantle fold; *oc*, eye; *op*, operculum; *osph*, osphradium; *ot*, otocyst; *ped*, pedal nervous cords; *per*, pericardium; *r.k*, right kidney; *r.s*, radula sac; *r.ur*, right ureter; *sal*, salivary gland; *st*, stomach; *v.l*, visceral loop of the nervous system.

result the skin on one side would be stretched and stimulated to grow, while the skin on the other side would be crushed and its growth inhibited.

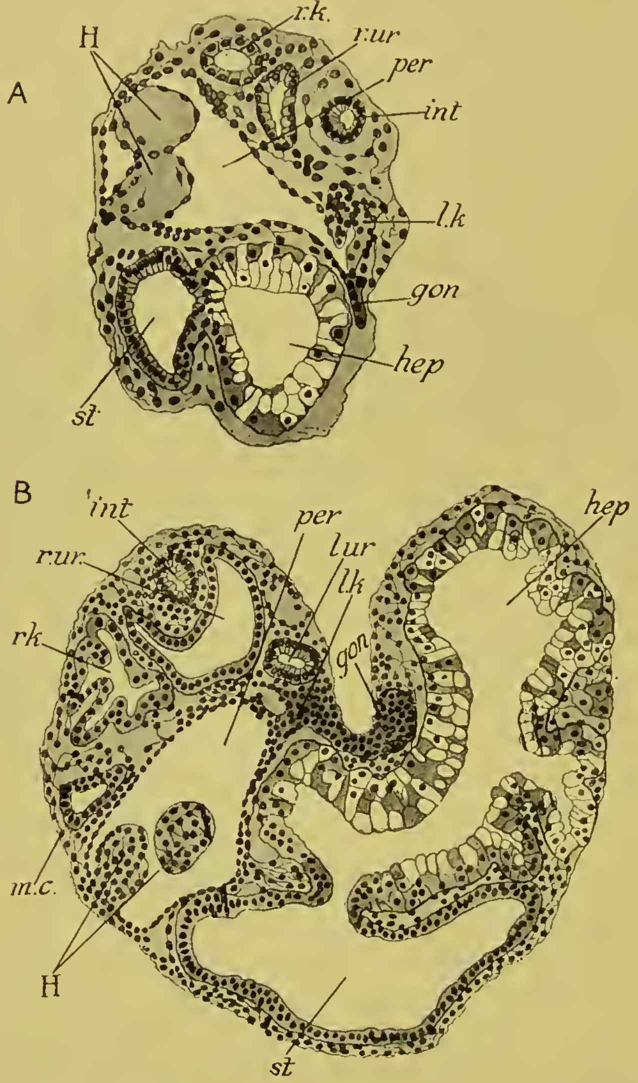

Fig. 252.—Transverse sections through the visceral humps of two embryos of *Paludina vivipara* of different ages to illustrate the torsion of the organs, the development of the genital organs and their connection with the right kidney, and the lengthening of the visceral hump associated with an increase in length of the liver. (After Drummond.)

A, younger stage in which the gonad is beginning as a thickening of the wall of the pericardium, and in which the left kidney is still distinct. B, older stage in which the gonad is connected with the rudiment of the left kidney. The right kidney has passed completely to the left. Letters as in previous figures.

Such is, in outline, the *organogeny* of a Mollusc; and, as mentioned above, Erlanger's work, except in so far as concerns the origin of the mesoderm, has been confirmed by the most recent workers on Mollusca.

METHOD OF RECONSTRUCTING ORGANS FROM SERIES OF SECTIONS

A word on certain methods is here advisable. The method of handling and making sections of minute embryos has been fully described in Chapter II.; but in the examination of sections, the observer, in looking through a series, mentally synthesises the pictures presented by successive sections, and thus forms a conception of the organ of which they each form part. This, with a little practice, is a comparatively easy matter when one is dealing with a bilaterally symmetrical animal or any animal whose body can be compared to a cylinder; when, however, one is dealing with an animal which is twisted in a spiral fashion, like *Paludina*, the mental synthesis is a very difficult matter, and, to assist it, the following method of reconstruction is followed.

The usual way of applying this method is to draw outline sketches of successive sections at a constant magnification, for example 200 diameters. If the sections are 5μ thick, *i.e.* $\frac{5}{1000}$ of a millimetre, which is a usual thickness in dealing with minute objects, then, such a section, being magnified in all its dimensions to the same extent as the picture that is drawn of it, would be 1 millimetre thick. The drawings are therefore transferred to wax plates 1 millimetre thick, and the outline of the particular organ whose course it is desired to study is boldly drawn in a conspicuous colour. Round this outline the wax plate is cut away; and the pieces of successive plates are piled on one another in the proper order, and in this way the solid form of the organ is reconstructed. In theory the pieces from successive plates should fit exactly, but in practice it is found necessary to adapt them to each other by melting the edges with a hot scalpel.

Prof. Graham Kerr has, however, elaborated a method by means of which practically as good results are obtained with infinitely less labour. Instead of wax plates he used square plates of fine ground glass of appropriate thickness. On these the outlines of successive sections of the organs which it is desired to reconstruct are drawn with a pencil of coloured chalk. The plates are now piled upon each other in proper order in a square glass vessel which is filled with cedar oil of the same refractive index as that of the glass used—both oil and plates being, of course, specially made for the purpose. The result of this arrangement is that the glass becomes absolutely invisible when immersed in the cedar oil, the coloured outlines of successive sections stand out boldly, and the solid form of the organ is conspicuous at the first glance. One great advantage of this method is that the materials, glass plates and cedar oil, can be used over and over again, since the pencil outlines are easily washed off.

OTHER GASTROPODA

We shall now examine how far the developmental history which we have described in *Patella* and *Paludina* is exemplified in the case of the other Gastropoda which have been studied. We find that those primitive forms which preserve the original bilateral symmetry (the Polyplacophora, *Chiton* and its allies) possess a typical Trochophore larva, similar in all respects, so far as our knowledge goes, to that of *Patella.*

The cell-lineage of *Ischnochiton,* as worked out by Heath (1899), seems to be exactly similar to that of *Patella,* and the formation of organs in the European *Chiton polii,* as described by Kowalevsky (1883), wears an even more primitive aspect than in *Paludina*; for example, the two pericardial sacs in *Chiton polii* are large, and occupy most of the post-trochophoral region. A re-examination of the later larval history of Polyplacophora by the aid of refined modern methods would be of rare interest.

A Trochophore larva is also found in the case of such primitive forms as *Trochus,* which has been worked out in great detail by Robert (1902), and in *Acmaea, Fissurella,* and *Haliotis,* all of which retain two auricles in the heart and two kidneys.

In all other Gastropoda, so far as is known, the embryo becomes a larva only when the Trochophore stage has been passed through and the coiled shell has been formed. Since, in almost every case, the eggs only develop after they have been laid, this involves their being laid in capsules secreted by the oviduct of the mother. Sometimes many eggs are contained in a capsule (Prosobranchiata generally), sometimes only one (Opisthobranchiata and Pulmonata), and in this latter case the capsules are very small and generally embedded in a jelly which is difficult to get rid of. The capsules of the Prosobranchiata are usually large, they are unprotected by jelly and attached singly to submarine objects such as stones. It is easy enough to detach them and slit them open, and in this way a supply of embryos can readily be obtained.

It is interesting to see in some such cases (cf. *Purpura*) the beginnings of the same process which has led to such distortions of development as are seen in the case of Platyhelminthes. Some of the embryos in a capsule develop imperfectly and are swallowed by their successful sisters, to whom they serve as pabulum.

In dealing with the egg-capsules of Pulmonata and Opisthobranchiata, which are immersed in jelly, various methods are employed. Sometimes, as in the case of the Opisthobranch *Fiona,* it is found possible to use reagents (for example, picro-acetic and picro-sulphuric acids) which will preserve the whole mass in bulk, and the jelly with the contained egg-capsules can then even be embedded in paraffin; but usually it is necessary to remove both jelly and capsules.

We may give Wierzejski's method of dealing with the eggs of *Physa* (1905) as a good example of how this can be accomplished. The egg-capsules are dissected out of the jelly by needles, then they are immersed for two or three minutes in a mixture of the solution of corrosive sublimate in water and glacial acetic acid, and the adhering jelly is then removed by the action of distilled water, in which it is soluble. The egg-capsule is then opened by a prick with a needle, and the embryo, as it floats out, is taken up in a pipette and put into 30 per cent spirit, which is after a time exchanged for 50 per cent spirit, and so by degrees the embryo can be immersed in absolute alcohol. This method is employed when it is desired to cut sections of the embryos.

For the study of cell-lineage, however, another method is preferred. The whole mass of jelly with its contained egg-capsules is thrown into a mixture of equal proportions of Perenyi's fluid and water. The jelly at first turns milky but gradually becomes clear. Then the capsules are opened by needles and the embryos float out. They are put into 15 per cent alcohol, and then into 30 per cent for twenty-four hours, and then gradually brought through higher grades of alcohol into absolute alcohol. The right moment to open the capsules must be carefully observed; if that time be allowed to pass the jelly turns milky again.

A method of staining with silver nitrate was also employed by Wierzejski, and when successful it caused the outlines of the cells to be indicated by brown lines. A ·75 per cent solution is used; in this the capsules are allowed to stay until they are brown, then they are opened and the embryos washed with alcohol. Wierzejski mounts his embryos in a mixture of balsam and clove oil, which remains sufficiently fluid to allow the embryos to be rolled about by the motion of the coverslip. The coverslip is supported by thin pieces of paper, or by thin pieces of glass tube drawn out to the proper degree of tenuity. By slight modifications of this method the eggs of all Opisthobranchiata and Pulmonata can be dealt with.

The development of a Gastropod in which the larval stage begins at the time when shell and foot have been formed, bears the same relation to that of *Patella* as the development of *Nereis* sustains to that of *Polygordius*. The most important differences are the extremely early indications of the future asymmetry, and the reduction of the prototroch. In *Crepidula*, which has been studied by Conklin (1897), and in *Fiona*, which has been worked out by Casteel (1904), for example, only the anterior primary trochoblasts (*i.e.* the descendants of $1a^2$ and $1b^2$) develop cilia, the greater part of the ciliated band being formed from "secondary trochoblasts," which include the tip cell $2b^{11}$ and descendants of $2b^{12}$ and $2b^{21}$, and even (in *Fiona*) of certain cells of the third quartette.

If this description is followed it will be seen that the velum consists principally of two anterior lobes; the circle is completed in *Crepidula* by a band of ciliated cells which runs across the anterior hemisphere of the larva in front of the apical cells, in *Fiona* by an ill-defined ciliated area extending over the posterior part of the front hemisphere, and in *Physa* not at all. When, as in many Prosobranchs, the state of affairs is as in *Crepidula*, the eyes and tentacles are excluded from the velar area, and hence the homology of the velum with the Annelidan prototroch has been seriously questioned; but when the structure of the embryos of *Patella*, *Trochus*, and *Chiton* was elucidated, the real homology of velum and prototroch became apparent.

Another difference is seen in the development of the "cross." This becomes far more conspicuous in the case of most Gastropods than it does in *Patella*. The terminations of its four arms are formed by the "tip cells" $2a^{11}$, $2b^{11}$, $2c^{11}$, and $2d^{11}$. The basal cells are of course $1a^{121}$, $1b^{121}$, $1c^{121}$, and $1d^{121}$, while tucked away between the four basals and the apical cells $1a^{111}$, etc., are the so-called **"peripheral rosettes"** $1a^{112}$, etc., which represent the **Annelidan cross,** but which do not divide more than once in Mollusca and hence do not attain any great development. The basal cells of the Molluscan cross, on the contrary, divide several times transversely, and then the daughter cells in the a, b, and c quadrants become longitudinally divided into two and even into four rows of cells (Fig. 252). In the d quadrant they remain undivided longitudinally for a considerable time, but also eventually divide, filling up the gaps between the apical and the prototrochal cells; the latter usually divide only once, forming eight **"turret cells,"** of which only the anterior, as we have seen, develop cilia. These points can be well seen in the segmenting egg of *Crepidula* (Fig. 253).

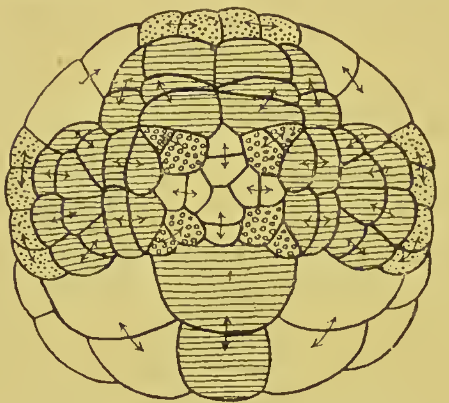

FIG. 253.—Apical region of an embryo of *Crepidula* showing the Molluscan cross in a late stage of development. (After Conklin.)

The apical cells are unshaded, as are also the primary trochoblast cells. The "peripheral rosette" cells (Annelidan cross) are marked with small circles. The cells of the Molluscan cross are ruled with horizontal lines, except the derivatives of the tip cells, which, since they belong to the second quartette, are dotted.

The most interesting thing that has been elucidated in the development of these more modified Gastropods is the relation of the organs of the veliger to certain groups of cells in the cell-lineage. Thus, in *Planorbis* it is found that the cerebral ganglia arise by internal proliferation from the lateral arms of the cross, except from their tip cells and the cells immediately adjoining these; from the anterior arms, except from their tip and basal cells; and from the two hinder arms of the Annelidan cross.

In *Crepidula*, *Physa*, and *Fiona* it is found that the mother cell of the mesoderm, 4d, divides as usual into right and left cells, $4d^r$ and $4d^l$. These then bud off two small anterior cells whose fate is to become endodermic, while the mesodermic mother cells divide into equal parts; so that we have four large mesodermic mother cells, two on each side. From each of the inner pair of mother cells a second small cell is given off; and these, with the first two cells, form a group of four small cells which lie close to the macromeres behind.

When the macromeres, by further division, have formed the larval stomach, these four cells seem to give rise to the hinder part of the intestine. They may be termed **mesendoderm.** There is no doubt at all that a similar state of affairs will be found in *Patella* when the cell-lineage has been fully worked out. It is another proof that the coelomic cells are essentially endodermic in origin.

The so-called **mesectoderm** or **larval mesoderm,** consisting of ectodermal cells which wander inwards and are converted into the muscles of the larval oesophagus, is derived in Mollusca generally from the anterior quadrants (a and b) of the third quartette.

The **stomodaeum** in *Fiona, Crepidula,* and *Physa* arises from $2b^{222}$ and $2b^{221}$ in front, and at the sides from cells of the third quartette, to a certain extent, as in *Polygordius.* In these Mollusca, however, these third-quartette cells succeed in excluding $2a^{222}$ and $2c^{222}$ from the sides of the stomodaeum, whilst behind it is closed entirely by third-quartette cells. In this case $2a^{222}$ and $2c^{222}$ give rise to lateral ridges of cells, between which and the opening of the mouth there are grooves filled up by third-quartette cells. In these grooves two pits appear (*Physa*) which form the rudiment of the radula sac. The ridges formed by $2a^{222}$ and $2c^{222}$ meet behind the mouth so as to enclose the pits and the mouth in a common **atrium** or outer stomodaeum; so that eventually $2a^{222}$ and $2c^{222}$ do form the outer stomodaeum.

The foot in primitive forms, such as *Trochus* and *Patella,* is at first double; it arises from descendants of 2d, in the region of the ciliated groove.

The most puzzling things about Gastropod Mollusca are the **larval kidneys.** These appear to be absent in Marine Prosobranchiata, but are found both in Pulmonata such as *Limnaea* and in fresh-water Prosobranchiata such as *Paludina.* In these forms they consist of V-shaped tubes with the apex of the V directed forwards, and they are formed of one huge giant cell. The internal end, *i.e.* the upper limb of the V, is a solenocyte; the lower limb opens to the exterior not far behind the head and a long way in advance of the opening of the permanent kidneys. At first sight one would be strongly inclined to regard these kidneys as equivalent to the archinephridia of Annelida. But the painstaking analysis of Wierzejski (1905) has conclusively proved that, in the case of *Physa* at any rate, the larval kidney arises from three cells at the anterior end of a row which is budded from the outer mesodermic teloblast on each side; and Erlanger (1894) found that in *Paludina* the larval kidney was segmented off from the anterior tip of the coelomic vesicle on each side. Therefore this type of kidney really belongs to the type which Goodrich terms **coelomiduct,** and its appearance would seem to indicate that Gastropoda originally possessed two pairs of coelomic kidneys—a conclusion which on other grounds may be regarded as extremely probable.

In Opisthobranchiata, on the other hand, there is found either one or a pair of dark excretory vesicles situated extremely far back in the neighbourhood of the anus. This vesicle, in *Aplysia*, has been supposed by Mazzarelli (1898) to be the rudiment of the permanent kidney, but Holmes (1900) declares that in *Fiona* the main part of it is formed from a large cell which he identifies as $3c^{111}$. In *Umbrella* Heymons (1893) finds a pair of these organs which arise from $3c^{11}$ and $3d^{11}$. They thus roughly correspond to the archinephridia of *Polygordius* in position.

The whole uncertainty in the matter arises from the fact that in no single species of Opisthobranchiata have we the complete developmental history of the organ from its earliest origin in the embryo until the larva has metamorphosed into the adult; and Comparative Embryology, so long as it is based on bits and scraps of development, is bound to be full of obscurities and apparent contradictions.

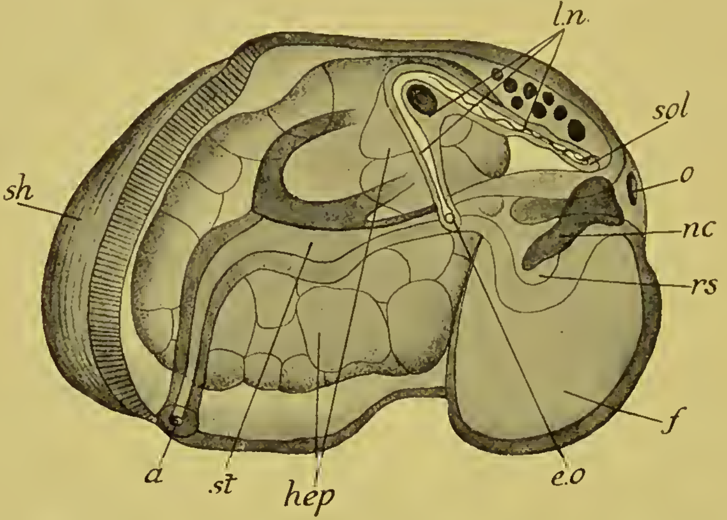

FIG. 254.—Embryo of *Limnaea stagnalis* viewed from right side as a transparent object in order to show the larval kidney. (After Erlanger.)

a, anus; *e.o*, external opening of the larval kidney; *f*, foot; *hep*, lobes of liver; *l.n*, larval kidney; *n.c*, nerve collar; *o*, mouth; *r.s*, radula sac; *sh*, shell; *sol*, solenocyte of the larval kidney; *st*, stomach.

In Marine Prosobranchiata there are frequently present two external protuberances of ectoderm cells, situated on each side and behind the velum. These become filled with excreta, and are eventually cast off. When it is remembered that the archinephridia of *Polygordius* owe their origin to ectodermal cells, it will be seen that it is quite possible to regard these external nephridia as homologous with them.

SOLENOGASTRES

We now pass on to consider the developmental history in other classes of Mollusca. The Solenogastres are an extremely primitive and at the same time a degenerate group, in some of which the ventral ciliated groove is retained throughout life; but they have been shown in one case to possess a typical Trochophore larva. This case is that of *Dondersia*, and the Trochophore is gradually converted into the adult form by the elongation of the post-trochal region.

SCAPHOPODA (*Dentalium*)

The Scaphopoda, with tubular shell and mantle, are represented by *Dentalium* and a few closely allied genera. The development of *Dentalium* has been worked out by Wilson (1904). It is practically of world-wide distribution, being found in muddy bottoms. Wilson found the eggs of the Mediterranean species ripe in June, and he gives the following description of them. They are yolky and deeply coloured by pigment which varies in tint from olive-green to brownish-red. When dehisced from the ovary the egg is almost as flattened as a biscuit, though one side is more flattened than the other, and this side is proved subsequently to be the vegetative pole. In the centre of each flattened surface is a white non-pigmented area.

After remaining in sea-water for from twenty to thirty minutes the egg becomes spherical and bursts its ovarian membrane or chorion. A jelly-like layer which surrounds the egg then swells up, and the egg now looks like a sphere with white poles and a broad ring of pigment. But when the egg is fixed in picro-acetic and cut into sections, the two poles are seen to be widely different. At the vegetative pole there is a dense mass of cytoplasm, devoid of yolk, which is continuous with a thin layer of clear cytoplasm surrounding the egg. This mass of cytoplasm also extends upwards through the egg to the germinal vesicle or nucleus, which is situated near the animal pole and surrounds it. At the animal pole there is a minute disc of cytoplasm free from yolk, which is far too small to account for the large white area seen in the living egg in this region. This latter must owe its appearance therefore to the presence of yolk granules of white colour.

As the egg lies in sea-water the cytoplasm of the animal pole slowly increases in amount, seemingly by a radial inflow from surrounding regions. The wall of the nucleus now breaks down and the first polar spindle is formed. Things are now at a standstill until the egg is fertilized, when the two polar bodies are formed one after the other. The spermatozoon enters at the vegetative pole. From this pole a pillar of granular cytoplasm extends upwards and becomes temporarily confluent with the cytoplasmic area at the upper pole. This pillar is in large measure produced by the material which was contained in the nucleus of the unripe egg, and which was extruded when the nuclear wall broke down.

The first cleavage occurs half an hour after fertilization, and is vertical. At the same time the lower white pole of the egg is cut off from the rest by a horizontal constriction. Sections show two things—first, that this lower sphere contains, besides the vegetative cytoplasm, a certain amount of yolk; and secondly, that it remains in connection with one of the two spheres produced by the vertical cleavage, by means of a thin pedicle never completely severed. As the cleavage of the two blastomeres from one another becomes complete, the lower sphere coalesces with one of the two upper spheres, and the blastomere so formed is shown afterwards to be CD. The lower sphere has been somewhat inappropriately named the **yolk lobe**, for

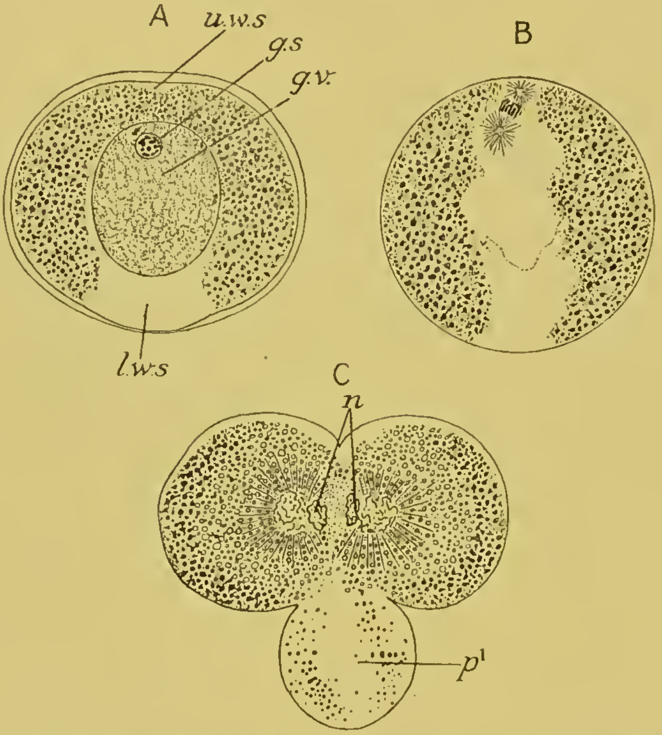

FIG. 255.—Vertical sections of the eggs of *Dentalium* before and after fertilization in order to show the flow of cytoplasmic substances. (After Wilson.)

A, before fertilization—after extrusion from the oviduct. B, after fertilization—formation of the first polar spindle. C, division into two blastomeres—extrusion of the first polar lobe. *g.s*, germinal spot, *i.e.* nucleolus; *g.v*, germinal vesicle; *l.w.s*, lower white substance; *n*, the first two daughter nuclei separating from each other; p^1, first polar lobe; *u.w.s*, upper white substance.

which Wilson substitutes the name **polar lobe**, and its fusion with one of the two blastomeres is known as the **retraction of the polar lobe.**

When the next cleavage occurs, AB of course divides into A and B, and the polar lobe is again constricted from CD, but at the conclusion of the cleavage it fuses with D, which is thus rendered by far the biggest of the first four blastomeres. A and C, moreover, as usual, meet in an upper cross furrow. Each of the four cells contains a portion of the white area which was situated at the animal pole of the egg, but only D has the white material of the vegetative pole.

At the next cleavage the first quartette of micromeres are given off. These consist entirely of the white material, though some of this still remains in the macromeres. The polar lobe is again constricted off from D, but it is much smaller than before and the constricting furrow does not extend so deeply. When the cleavage is complete the polar lobe again fuses with 1D.

Before the next cleavage occurs the white material derived from the animal pole, part of which was left in each macromere, increases in amount, moves over to the right side of each cell, and extends somewhat down the side. In 1D this also occurs, but in this cell the white material from the animal pole is joined by the white material from the vegetative pole, which moves over and fuses with it. Of the second quartette of micromeres 2d is formed first, and it is composed of the white material derived from both poles, whereas 2a, 2b, and 2c, which are formed soon afterwards and likewise consist of white material, have received only white material which was originally at the animal pole of the egg. At the same time the first-quartette cells divide into the trochoblasts ($1q^2$) and the upper cells, the latter

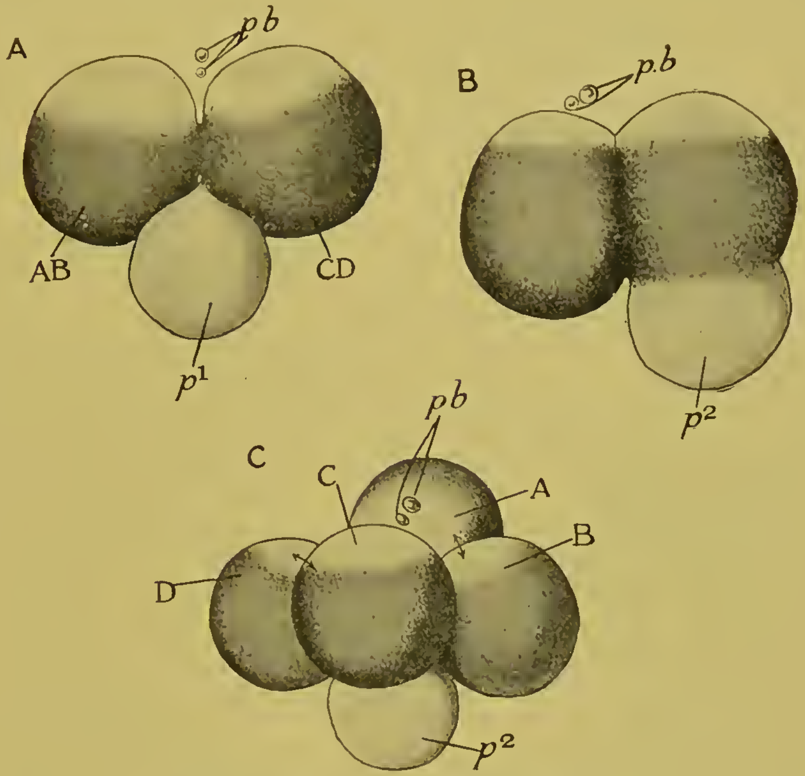

FIG. 256.—Stages in the cleavage of the egg of *Dentalium*. (After Wilson.)

A, completion of the first cleavage. B, beginning of the second cleavage, seen from the side. C, the second cleavage in its most intense period, seen obliquely from above and the side. p^1, the first polar lobe; p^2, the second polar lobe; *p.b*, polar bodies.

being slightly larger. The third quartette is formed as usual at the next cleavage; 3d is larger than its sisters, and entirely composed of white material.

After this cleavage gastrulation begins by the macromeres passing bodily into the blastocoele, just as in *Patella*. Of the fourth quartette 4d alone was clearly observed; it is smaller than 3d and very much smaller than 2d, and is *pure white*.

As in *Patella*, cilia are developed about ten hours after fertilization, and in twenty-four hours well-developed Trochophore larvæ are set free. These are remarkable for their very broad **prototroch**, which consists of three complete circles of large cells with cilia. The pre-trochal region is short and conical; it is covered all over with short cilia, and it bears at its apex an apical plate with a long tuft of motionless but flexible cilia. The post-trochal region is also short and conical, and at its posterior end there is a telotroch consisting of a tuft of short rigid hairs.

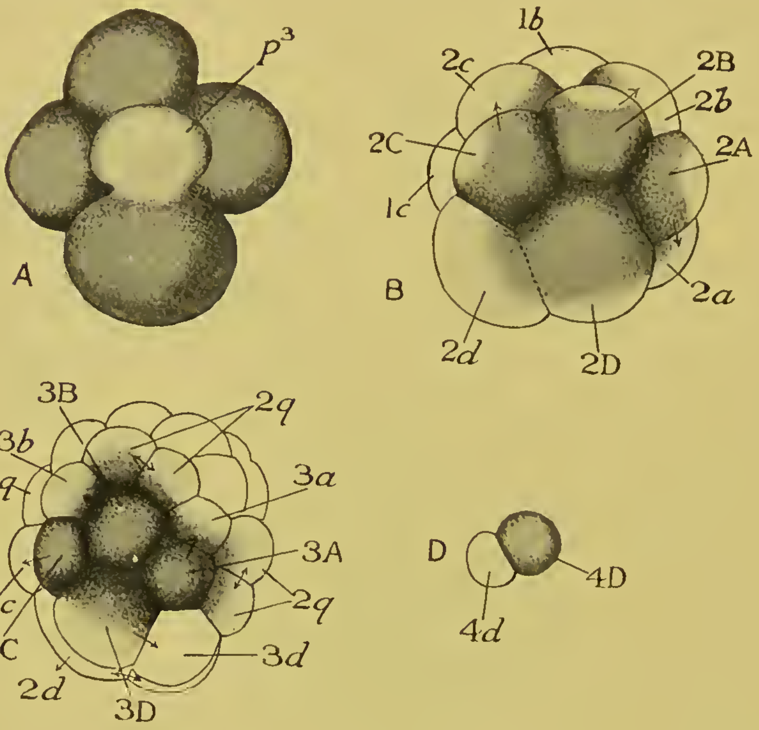

FIG. 257.—Further stages in the cleavage of the egg of *Dentalium*. (After Wilson.)

A, beginning of third cleavage (8-blastomere stage), seen from the lower pole. B, the formation of the second quartette of micromeres, seen from the lower pole. The greater part of the substance which formed the polar lobes passes into 2d. C, the formation of the third quartette of micromeres, seen from the lower pole. D, the division of the macromere which gives rise to the mother cell of the mesoderm. p^3, third polar lobe.

The **stomodaeum** has not yet opened into the gut. This latter consists of a sac-like **stomach** and a short blind **intestine**; the **anus** is not yet formed. At the sides of the intestine are seen two short **mesodermal bands.** In the pre-trochal region two masses of cells are seen lying to the right and left. These are proliferated from the ectoderm, and are almost certainly the beginnings of the **cerebral ganglia.** Very soon after the beginning of larval life the rudiment of the **shell gland** can be made out, and the everted edge of this already foreshadows the future **mantle fold,** which is at first double, like that of a Pelecypod.

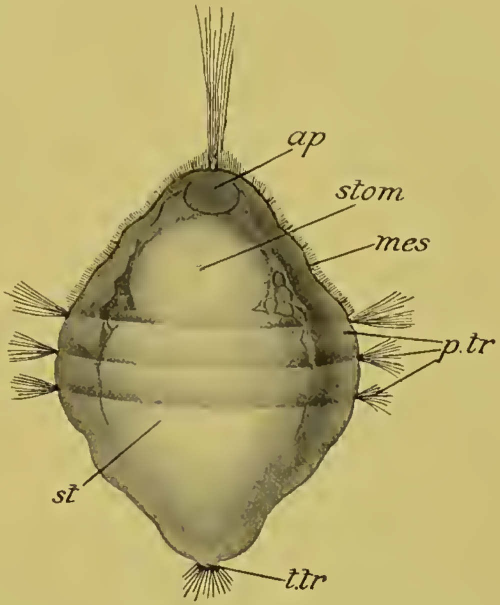

Fig. 258.—The Trochophore larva of *Dentalium*—twenty-six hours after fertilization. (After Wilson.)

a.p, apical plate ; *mes*, mesoderm ; *p.tr*, prototroch ; *st*, stomach ; *stom*, stomodaeum ; *t.tr*, telotroch.

During the course of the next day the larva sinks to the bottom ; the pre-trochal or velar region becomes relatively smaller whilst the post-trochal region grows very much in length, and then the velar region becomes finally completely invaginated, and in this way the larva attains the stage of a **veliger.**

By the end of the second day not only is the shell gland everted but a delicate hyaline **shell** has been formed, and into this the diminished prototroch or velum can be withdrawn. The **foot** has now made its appearance as a median ridge. At the end of the third day the foot has become large, protrusible, and bilobed at its free end ; and the mantle lobes have partially united beneath the animal. By the fifth day the prototroch has disappeared and the **otocysts** and **pedal ganglia** can clearly be seen ; the metamorphosis may now be said to be complete. It is worthy of note that the northern species of *Dentalium*, which was studied by Lacaze-Duthiers, took twenty-five days to reach the same stage.

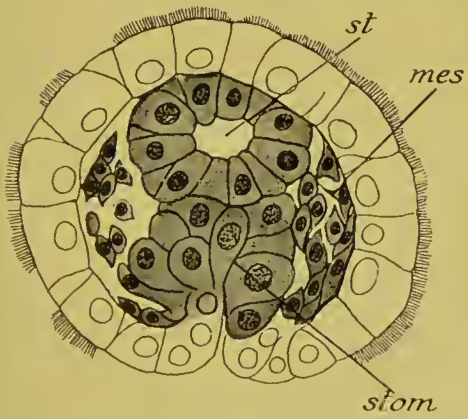

Fig. 259.—Transverse section of the Trochophore larva of *Dentalium* in the region of the prototroch. (After Wilson.)

Letters as in previous figure.

EXPERIMENTAL EMBRYOLOGY OF DENTALIUM

This peculiar development offers abundant opportunity for experiment, as Wilson was not slow to perceive. Some of these experiments were quite similar to those which he performed on *Patella*, and led to similar results; but the most interesting results were those obtained by removing the polar lobe, which can be readily done by means of a fine scalpel. When this is done at the time of the first cleavage the embryo continues to develop, but all the cells at the second cleavage are equal in size and possess no lower white area, and no polar lobe is subsequently formed.

At the subsequent cleavages the micromeres given off in the D quadrant are precisely similar in size to their sisters, and the embryo becomes a larva with a normal prototroch and a conical pre-trochal region; but there is no projecting post-trochal region, the posterior surface of the larva being almost flat. The pre-trochal region is covered, as normally, with fine cilia, but the apical tuft is absent, and so is the thickened apical plate which is present in normal larvae. On the other hand, the lateral ingrowths of ectoderm, which we suppose to represent the cerebral ganglia, are present.

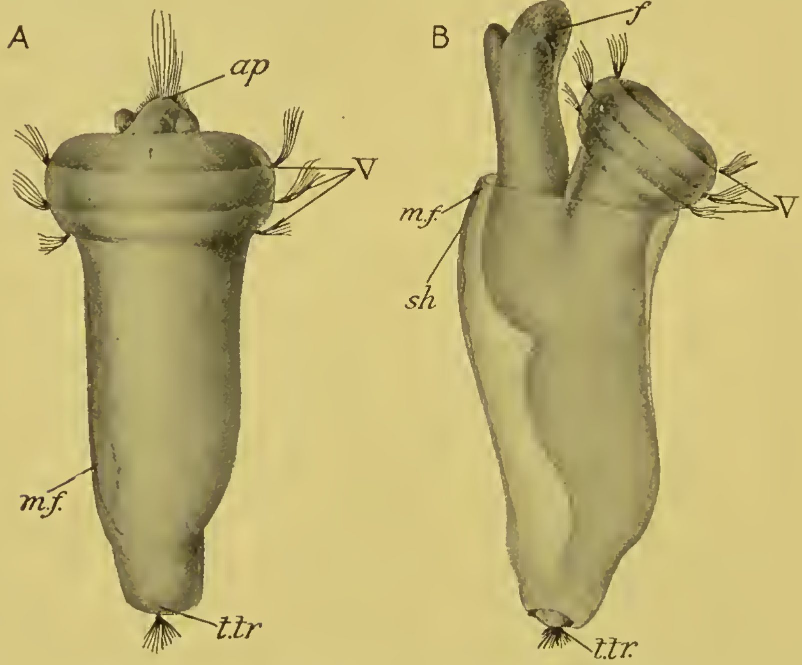

FIG. 260.—Veliger larvae of *Dentalium*. (After Wilson.)
A, Veliger larva, thirty-two hours old. B, Veliger larva, three days old. Letters as in previous figure. In addition, *f*, foot; *mf*, mantle fold; *sh*, shell; V, velum.

Such larvae live four days and then disintegrate. Occasionally a post-trochal protuberance appears to be formed, but when this is examined by sections it is seen to be a plug of solid endoderm projecting through the open blastopore. No mesodermal bands are ever seen. The conclusion is therefore inevitable that the first polar lobe contains the material necessary not only for the formation of the whole post-trochal region, but also for the formation of the apical plate. It must also contain the material necessary for the formation of the mesodermic bands.

This conclusion is confirmed by separation of the first two blastomeres; both of these, when isolated, continue to segment as if they formed part of a whole egg, but both subsequently give rise to larvae which swim about, though they possess a confused irregular prototroch. The larva derived from AB, however, in its general structure, resembles the larva developed from a whole egg from which the first polar lobe has been cut off, because it has neither apical plate nor post-trochal region. The larva derived from CD, on the other hand, which carries the polar lobe, though it is asymmetrical and has too small a pre-trochal region, has too large a post-trochal one and possesses a well-defined apical plate.

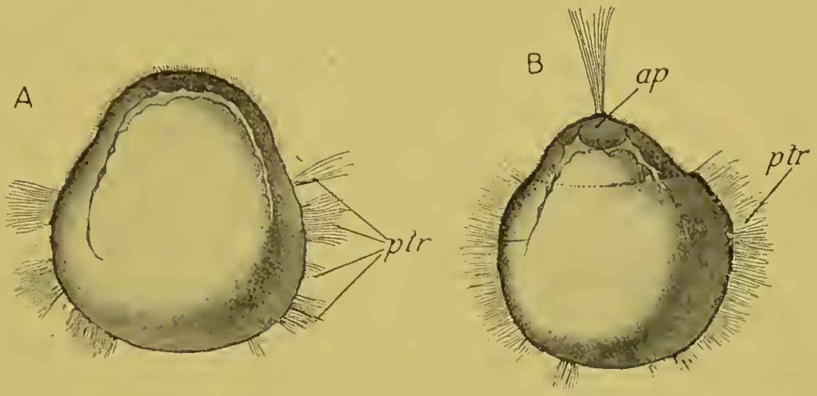

FIG. 261.—Larvae resulting from the development of eggs of *Dentalium* from which the polar lobe has been removed. (After Wilson.)

A, larva, twenty-four hours old, developed from egg from which the first polar lobe has been removed. B, Larva, twenty-four hours old, developed from egg from which the second polar lobe had been removed. Letters as in Fig. 258.

If the egg be allowed to reach the 4-cell stage, and if the polar lobe that is then protruded, that is the second one, be removed, a larva is produced in most respects similar to the one which arises from an egg from which the first polar lobe is removed; it possesses neither mesodermic bands nor post-trochal region, but it possesses an apical plate and the characteristic apical tuft of cilia. Therefore the second polar

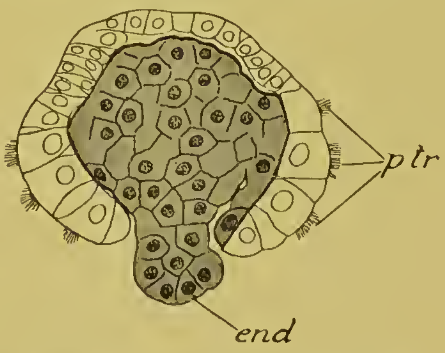

FIG. 262.—Vertical section of a larva of *Dentalium* developed from egg from which the first polar lobe had been removed, to show the absence of mesoderm. (After Wilson.)

end, protruding plug of endoderm.

lobe does not contain the specific organ-forming material for the apical plate; in the interval between the formation of the first and second polar lobes it has been distributed to a different region of the egg.

Where that region is it is not difficult to determine. If the micromeres of the first quartette be separated from each other by allowing the embryo to develop in artificial sea-water devoid of calcium, then each micromere will develop into a closed ectodermic vesicle; but only the micromere 1d develops an apical plate, and the apical "stuff" is therefore transferred to this micromere. Now, in *Patella* the apical plate is formed in larvae developed from each of the four micromeres of the first quartette; we have therefore in the development of *Dentalium* a case of specialization, similar to that which we often meet with in eggs with spiral cleavage, in which one member of a quartette does the work normally undertaken by all the sisters in other species. A case of this kind was met with in the first case of spiral cleavage which was studied, namely, in the development of *Planocera* as compared with that of other Polyclade Platyhelminthes.

These remarkable experiments of Wilson establish in the most incontrovertible manner the existence of specialized **organ-forming substances** in the egg of *Dentalium*. It is but fair to add that the first experiments of this kind were made by Crampton (1896) on the egg of the Gastropod *Ilyanassa*, where a similar polar lobe is found.

PELECYPODA—*Dreissensia*

We must now consider the development of that great group of Mollusca familiarly known as bivalves and scientifically as Pelecypoda or Lamellibranchiata. The most complete and satisfactory study of the development of any form belonging to this group is that by Meisenheimer (1901) on the life-history of *Dreissensia polymorpha*. This type we may therefore select for more special study.

Dreissensia is a genus found in brackish and fresh water both in England and on the continents of Europe and America. In form it closely resembles the marine genus *Mytilus*, the common mussel, to which it is regarded by many authorities as nearly allied, and from which it differs in having the two mantle lobes firmly united for a part of their length in the mid-ventral line, and in having the posterior opening prolonged into two separate tubular siphons. It is interesting from the fact that, though a fresh-water species, it retains a long larval development of very primitive facies, whereas most fresh-water species have a shortened, modified, and mainly embryonic development. *Dreissensia* is clearly a recent immigrant into fresh water.

Meisenheimer obtained his material from one of the small fresh-water lakes of Germany (the Plöner See). The eggs of *Dreissensia polymorpha* are laid in June, and are cast forth from the mother in masses, bound together with a slight amount of slime which is easily washed away. The eggs have no chorion of any kind, and hence are quite easily preserved. For the earlier stages corrosive sublimate and picro-sulphuric acid were the reagents used, but for the later stages and for the free-swimming larvae Hermann's mixture of osmium tetroxide, platinum chloride, and acetic acids gave the best results. It was necessary to paralyse the larvae by cautiously adding cocaine to the water in which they lived, before attempting to preserve them, otherwise they contracted themselves into shapeless lumps in which the natural relationship of the various organs could not be made out. The larval stages swarmed in the lake and were captured by using a fine-meshed Plankton net, so that the difficulties connected with artificial rearing were entirely avoided.

A striking feature of the early development of *Dreissensia* is the intermittent appearance of the **blastocoele.** This cavity is large and well developed in the 2-cell stage (Fig. 263); it subsequently disappears, but reappears in later stages, such as the 8-cell and 16-cell stages. Meisenheimer supposes that the blastocoele serves as a reservoir of excreta which are periodically voided.

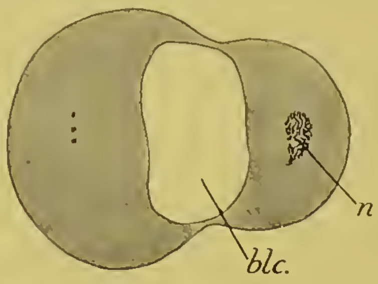

FIG. 263.—Longitudinal section of the 2-cell stage of *Dreissensia polymorpha* to show the blastocoele. (After Meisenheimer.) *bl.c*, blastocoele; *n*, nucleus.

The egg divides into the usual four macromeres A, B, C, and D, but of these D is so much larger than the rest that the remaining three appear much like micromeres budded from one large macromere. This state of affairs is worth bearing in mind in view of the extraordinary statements which have been made about the development of other Pelecypoda. When the first quartette of micromeres is formed, 1d appears first and is the largest, though the disparity in size between it and its sisters, 1a, 1b, and 1c, which appear subsequently, is not great. But at the next cleavage, when these micromeres divide, each into two daughters of equal size, and when the second quartette of micromeres is formed, one of these latter, 2d, is relatively enormous in size; it overshadows not only all the micromeres belonging to the first and second quartettes but its own sister macromere, 2D. This huge "micromere" corresponds to the one which Wilson, in the development of *Nereis*, has termed the **first somatoblast,** from which most if not all the ectoderm covering the body of the adult worm is derived.

From the first somatoblast of *Dreissensia*, termed by Meisenheimer X, is derived the shell-gland, and we have strong reason to suggest, although this is not quite proved, the foot; it has, however, been proved by Lillie (1895) in the case of *Unio*. The first somatoblast now gives off a cell below and to the right. This cell is of course $2d^2$: it is denominated by Meisenheimer x_1, since he calls the parent somatoblast X. At the next cleavage all the daughters of the first quartette divide again, so that we get four concentric circles of cells, $1q^{11}$, $1q^{12}$, $1q^{21}$, and $1q^{22}$.

The third quartette of micromeres now begins to be formed, 3d being formed before its sisters. X gives rise to a small cell on the left, the proper title of which is $2d^{12}$, but which is called by Meisenheimer x_2. The somatoblast has thus acquired at its lower border a wreath of three cells, x_1, 3d, and x_2. Of the second quartette of micromeres, which should have divided when the third quartette was being formed, only one (2d = x) has as yet divided. Another member of this quartette now divides, *i.e.* 2c; whilst $2d^2$ divides into $2d^{21}$ and $2d^{22}$—or, according to another notation, x_1 gives x_{11} and x_{12}.

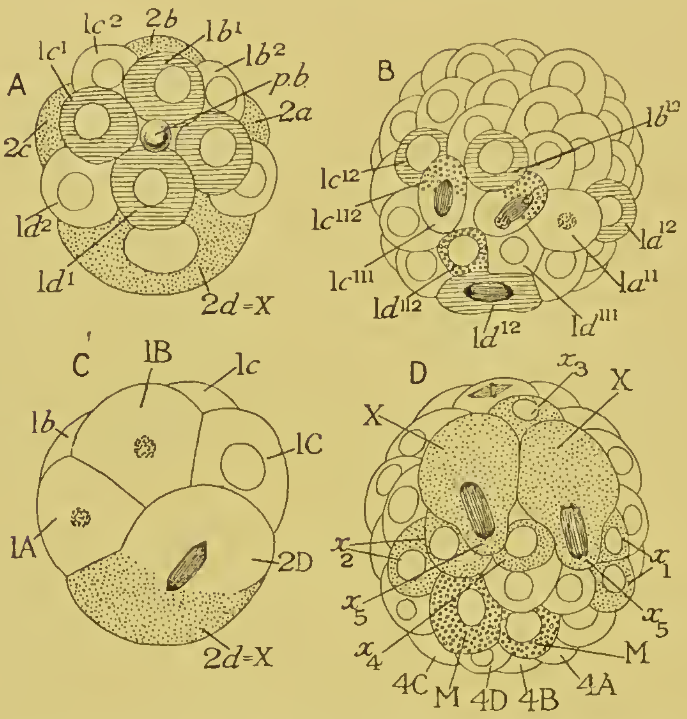

FIG. 264.—Stages in the cleavage of the egg of *Dreissensia polymorpha*. (After Meisenheimer.)

A, upper hemisphere of egg in the 16-cell stage. B, upper hemisphere of egg just passing into the 54-cell stage. The formation of the apical cells is seen. C, egg seen from the vegetative pole in the 8-cell stage at the moment when 2d is being formed. D, Posterior view of egg in a somewhat later stage than that shown in B, to show the primary mesoderm cells and some of the products of the division of X. M, primary mesoderm cell; *p.b*, polar body.

It is quite clear therefore that in *Dreissensia*, unlike *Patella*, the radial symmetry of the spiral type of cleavage is very early interfered with, and that the prospective importance of the organs derived from 2d is reflected back into a very early stage of ontogeny; this is testified to by the precocious divisions and development of the cells derived from this blastomere.

The remaining members of the third quartette of ectoderm cells, 3a, 3b, and 3c, are now budded off from their respective macromeres. Only after this has happened do the anterior cells of the second quartette, viz. 2a and 2b, divide into $2a^1$ and $2a^2$, $2b^1$ and $2b^2$, respectively; whilst the somatoblast X buds off from its upper border a small cell x_3.

All the cells of the first quartette now undergo renewed cleavage, so that we have eight circles of cells, viz. $1q^{111}$, $1q^{112}$, $1q^{121}$, $1q^{122}$, $1q^{211}$, $1q^{212}$, $1q^{221}$, and $1q^{222}$. In these divisions the members of each circle belonging to the D quadrant divide before their sisters. We have thus in *Dreissensia* the same typical divisions of the cells of the first quartette which are found in *Patella* and *Polygordius*; but Meisenheimer does not refer to or figure any conspicuous cross-like arrangement of any of these cells; on the contrary, he seems to imply that they continue to have a concentric arrangement.

At the lower pole of the egg a single representative of the fourth quartette is now given off. This is 4d, which Meisenheimer calls the "**second somatoblast**"; but it is of course homologous with the mother cell of the mesoderm in both *Polygordius* and *Patella*. At first the mother cell of the mesoderm, which we may designate as M, touches the second somatoblast; but the latter gives off a cell towards the vegetative pole which Meisenheimer calls x_4, and this, along with x_1 and x_2, completely separates X and M (Fig. 264, D).

After a few more divisions in the cells of the first quartette, the first unmistakable traces of bilateral symmetry make their appearance by the division of both X and M into right and left halves. Then from each half of X a small cell, x_5, is budded off posteriorly, and the arrangement of the derivatives of the first somatoblast is as shown below, viz.:

$$X - x_3 - X;$$

$$x_2 - x_5 - x_4 - x_5 - x_1.$$

Each half of M also buds off a small cell, and then, by repeated transverse divisions, a longitudinal plate of large cells which is the rudiment of the **shell gland** is developed out of the two halves of X.

Following the stage which we have just described, the process of gastrulation begins. The residual macromeres 4A, 4B, 4C, and 4D sink inwards towards the blastocoele. The small cells given off from the mother cells of the mesoderm sink in with them and go to build up the wall of the mid-gut. The mother cells should therefore be termed **mesendoderm**, not true **mesoderm**; they themselves lie posterior to the lip of the blastopore, and are partly invaginated with the endoderm in the process of gastrulation. By repeated division they give rise later (just as in *Paludina*) to a loose mesenchymatous mesoderm, out of which the connective tissue and muscles of the adult bivalve are formed.

The invagination of the mid-gut cells proceeds at first very slowly, because their progress is impeded by the much more rapid and conspicuous invagination of the cells forming the shell gland. This latter deep invagination lasts only a short time. Soon the cells forming the shell gland are again everted and form, as in *Patella*, a saddle-shaped plate with thickened edge, on the dorsal surface of the larva. On this plate a thin horny secretion, the first rudiment of the shell, appears. As the process of eversion takes place the invagination of the endoderm goes on rapidly, and soon a sac is formed whose wall is composed of large columnar cells, and which opens to the exterior by a constricted opening, the **blastopore** (Fig. 265).

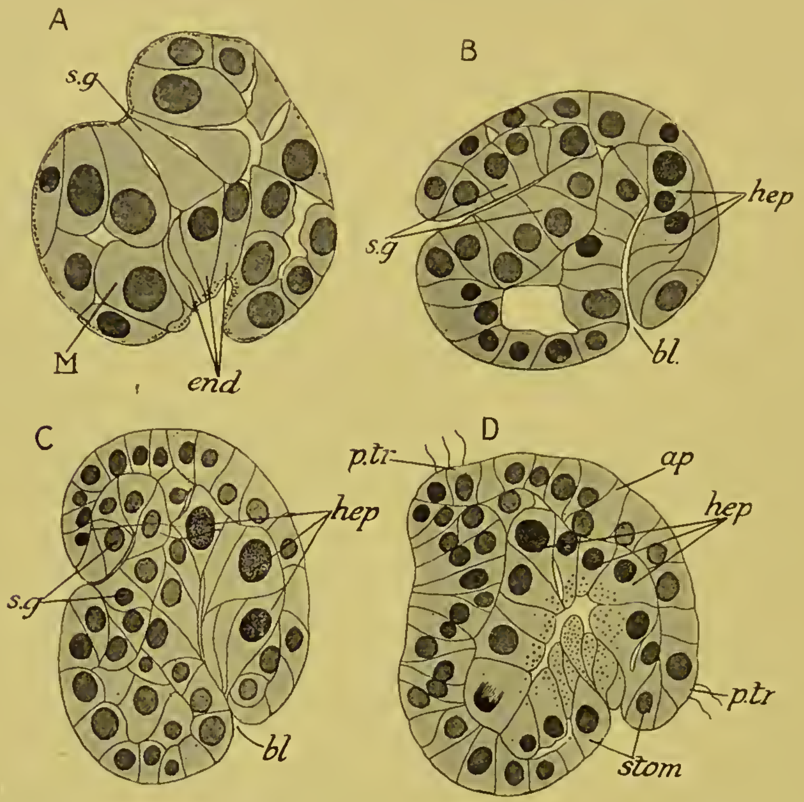

FIG. 265.—Sagittal sections of embryos of *Dreissensia polymorpha*, showing the process of gastrulation and the formation of the shell gland. (After Meisenheimer.)

A, stage in which the endoderm and the shell gland are both beginning to be invaginated. B, stage in which the invagination of the shell gland has reached its maximum. C, stage in which the blastopore is closed and the shell gland is beginning to be evaginated. D, stage in which the shell gland is completely evaginated and the stomodaeum is beginning to be formed. *a.p*, apical plate; *bl*, blastopore; *end*, endoderm; *hep*, cells which will eventually form the liver; M, primary mesoderm cell; *p.tr*, prototroch; *s.g*, shell gland; *stom*, stomodaeum.

The blastopore becomes shifted forwards and finally closed in the position where the mouth afterwards opens. This forward shift seems to be largely due to the growth of the band of small cells, $x_1 - x_5$, derived from X, which separated originally X and M on the posterior surface of the embryo. This band thus comes to occupy the region immediately behind the mouth; and as the foot is later developed in this region, it probably owes its origin to these cells. When the blastopore has been completely closed, the **stomodaeum** originates as an ectodermic invagination just where the last trace of the blastopore was situated.

The wall of the mid-gut, after the blastopore has become closed, undergoes a characteristic differentiation. The cells forming its anterior wall acquire large clear nuclei with conspicuous nucleoli, whilst those forming the lateral and posterior walls retain small deeply staining nuclei. Soon the peculiar cells of the anterior wall become confined to two slight outpouchings of the wall of the stomach, to the right and the left of the mid-ventral line. These pouches of the larval stomach will eventually give rise to the adult **liver.** From the posterior wall of the stomach is developed the **intestine,** and this grows backwards and becomes attached to the ectoderm behind the mouth. Here a very shallow invagination is formed, the **proctodaeum**; and at a slightly later stage, by the union of the proctodaeum and intestine, the **anus** becomes opened. In front of the anus is formed the **teloroch,** consisting of a couple of cells carrying stiff hairs.

So far we have not mentioned the **prototroch** and the **apical plate.** Both these structures appear about the time when the shell gland is everted; the prototroch is in the form of a girdle of cells carrying powerful cilia, and the apical plate in the form of a group of cells at the animal pole bearing a wisp of long stiff cilia. The cell-lineage of the cells forming these organs Meisenheimer was not able to determine, but there is no reason to doubt that it is, in the main, the same as in *Patella.* The prototrochal cells develop vacuoles in their interior, as is the case with the prototrochal cells of *Polygordius.*

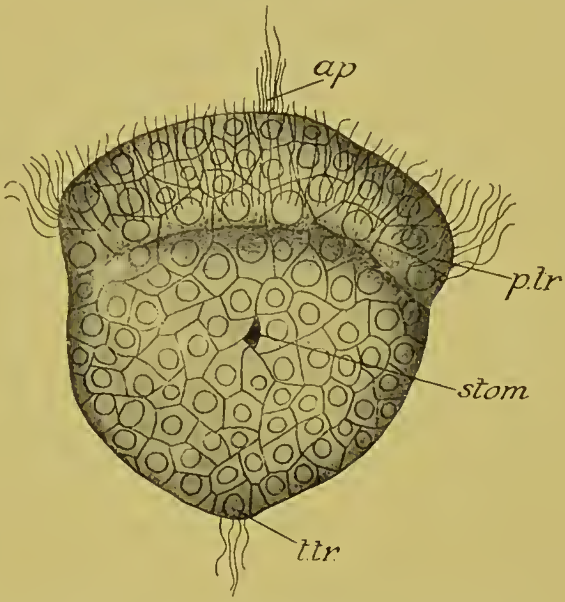

FIG. 266.—Young Trochophore larva of *Dreissensia polymorpha*, seen from the ventral side. (After Meisenheimer.)

Letters as in previous figure. In addition, *t.tr*, telotroch.

Lastly, situated just behind the spot where the anus will develop, there is a group of small cells which Meisenheimer believes to be of ectodermal origin, which will give rise, at a later period, to the **coelomic sacs** and to their derivatives, the **kidneys** and **genital organs.** This cell-group occupies precisely the same place as does the first rudiment of the pericardium in *Paludina*, and as do the mother cells of the mesoderm in an earlier stage of development in *Dreissensia.* When this stage of development has been attained, the embryo bursts the egg-membrane and enters on its free-swimming life as a Trochophore larva (Fig 266).

In *Physa*, as we have already seen, Wierzejski has traced the pericardium back to its origin in the derivatives of the mother mesoderm cells, through an unbroken series of stages. For these reasons we reject Meisenheimer's view of the origin of these cells, and believe that they are derived from the mother mesoderm cells after the latter have given off the mesenchymatous tissue alluded to above. This view would bring the development of Pelecypoda into harmony with that of other Mollusca, and should be definitely tested.

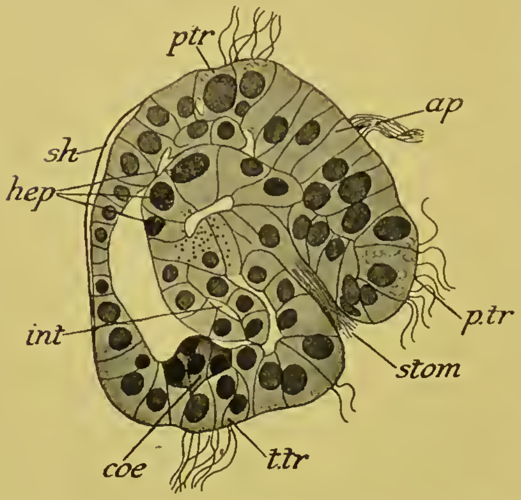

Fig. 267.—Sagittal section through a young Trochophore larva of *Dreissensia polymorpha*. (After Meisenheimer.)

Letters as in Figs. 265 and 266. In addition, *coe*, group of cells from which the coelom (pericardium) later develops; *int*, intestine; *sh*, primary shell (the adult hinge).

The Trochophore larva soon passes into the condition of a **Veliger** larva. This change takes place by the enlargement of the prototroch into the velum and by the growth of the bivalve shell. Behind the prototroch several rows of large cells are differentiated; they are covered with numerous fine cilia and reinforce the action of the prototrochal girdle; this enlarged

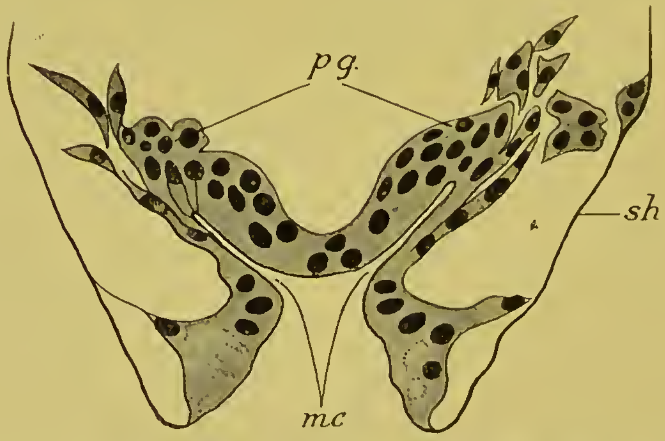

Fig. 268.—Transverse section of the ventral portion of a young Veliger larva of *Dreissensia polymorpha* to show the origin of the *mantle-groove* and of the *pedal ganglia*. (After Meisenheimer.)

m.c, mantle-groove; *p.g*, thickenings of ectoderm which will give rise to the pedal ganglia; *sh*, shell.

structure is now known as the **velum**. These additional cells remind us of some of the "secondary trochoblasts" of *Patella*.

The **shell** of the Trochophore is merely a thin horny cuticle secreted by the cells of the everted shell gland. This cuticle adheres closely to the ectoderm in the mid-dorsal line, and the ectoderm cells here become columnar; this region constitutes the **hinge** of the adult shell. The cuticle adheres loosely towards the sides, but at the edges of the shell gland a renewed deposition of cuticle takes place in two small circular areas which rapidly extend in dimensions, and in this way the valves of the **bivalve shell** are laid down. Not merely horny, but also calcareous material is secreted by these shell-forming areas.

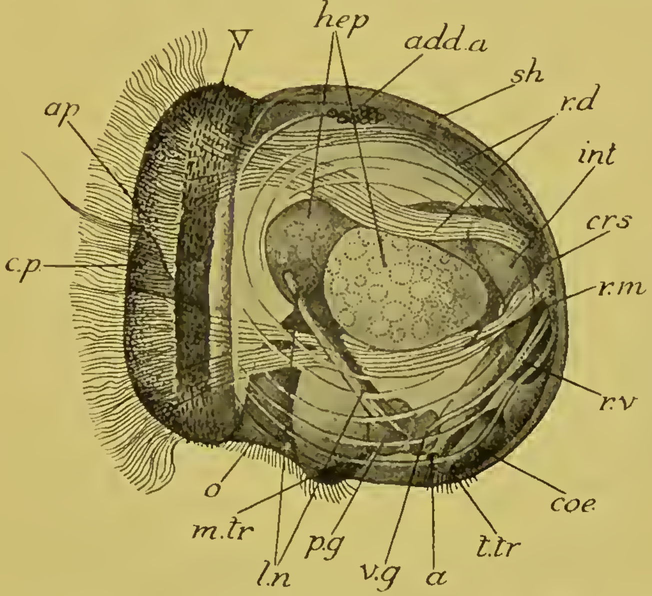

FIG. 269.—Young Veliger larva of *Dreissensia polymorpha*, seen from the side. (After Meisenheimer.)

a, anus; *add.a*, adductor muscle; *a.p*, apical plate (afterwards becomes the anterior adductor muscle); *coe*, rudiment of coelom; *c.p*, cerebral pit; *cr.s*, crystalline sac; *hep*, lobes of liver; *int*, intestine; *l.n*, larval kidney; *m.tr*, metatroch; *o*, mouth; *p.g*, pedal ganglion; *r.d*, dorsal retractor muscle; *r.m*, middle retractor muscle; *r.v*, ventral retractor muscle; *sh*, shell; *t.tr*, telotroch; *v.g*, visceral ganglion; V, velum.

The larva now changes its shape and instead of being cylindrical becomes more or less laterally compressed. As the newly formed shell valves extend towards the mid-ventral line, the **mantle-cavity** appears as two longitudinal invaginations on the ventral surface (Fig. 268). By the appearance of these grooves the edges of the area formed from the everted shell gland are changed into right and left mantle-lobes. The valves of the shell have a characteristic shape which appears to be practically universal amongst the veliger larvae of Pelecypoda. The hinge-line is straight and horizontal and the lower margin of the valve is curved, so that the shape of the whole may be described as semicircular (Figs. 269, 271).

Behind the mouth, which is situated on a projecting oral cone, is a post-oral tuft of cilia, the sole representative of the **metatroch** of Annelida. The oesophagus is ciliated, and small organisms are whisked into the stomach. The modification of the anterior wall of the stomach into the liver-pouches has already been mentioned. From its posterior ventral wall a short pouch grows out on the left side, whose cells secrete rod-like excrescences. This is the rudiment of the **crystalline sac** which secretes the **crystalline style** (Figs. 269, 270).

A **larval kidney**, consisting of a straight tube opening at the side of what is afterwards the foot, and terminating internally in a solenocyte situated near the liver-pouch, makes its appearance at the same time as the shell and disappears as the foot grows out. Meisenheimer derives it from an ingrowth of the ectoderm, but he has no convincing evidence to prove this. We think it more probable that it arises, as Wierzejski (1905) has proved that it arises in *Physa*, from cells budded off from the mother mesoderm cells.

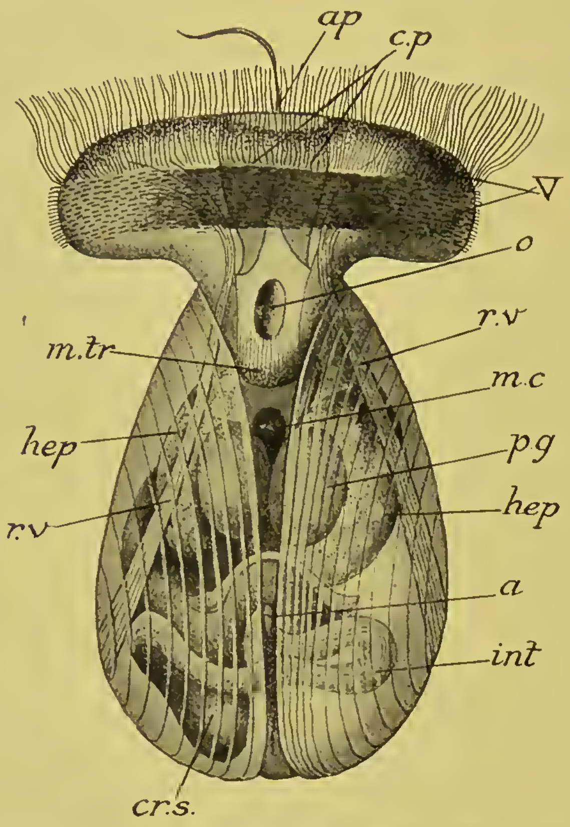

FIG. 270.—Young Veliger larva of *Dreissensia polymorpha*, seen from the ventral surface. (After Meisenheimer.)

Letters as in previous figure. In addition, *m.c*, mantle-cavity.

Three sets of powerfully developed muscles are formed, consisting of spindle-shaped cells which arise from the proliferation of the mesoderm cells. All three are inserted into the cuticle of the hinge in the posterior dorsal region, and all pass forwards and slant downwards. The uppermost of these is the **dorsal retractor,** the fibres of which pass forwards and diverge to the right and left and are inserted into the upper parts of the velum; below it lies the **median retractor,** which sends fibres to the lateral and ventral parts of the velum; whilst below this again lies the **ventral retractor,** which is inserted into the anterior portions of the right and left mantle-lobes. All these muscles are of a transitory character and disappear when

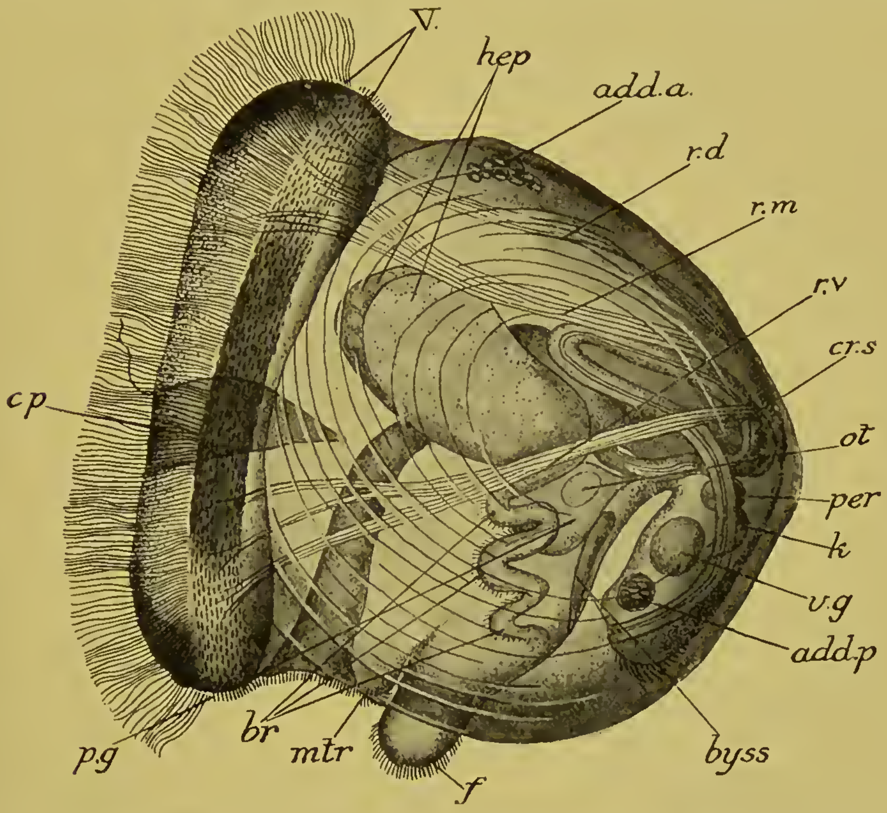

Fig. 271.—Older Veliger larva of *Dreissensia polymorpha*, seen from the side. This stage is the one which immediately precedes the metamorphosis. (After Meisenheimer.)

Letters as in two previous figures. In addition, *add.a*, anterior adductor muscle; *add.p*, posterior adductor muscle; *br*, rudiments of gill-papillae; *byss*, byssus gland; *k*, rudiment of kidney; *ot*, otocyst; *per*, rudiment of pericardium.

the free-swimming life is given up; but the **anterior adductor muscle,** passing from one valve of the shell to the other, is already formed at this stage, and it persists into the adult.

The three pairs of ganglia characteristic of Mollusca make their appearance at this stage. Of these the **cerebral ganglia** owe their origin to a bilobed pit, termed the **cerebral pit,** situated within the velar area in front of and below the ciliated apical plate. This pit evidently corresponds to the two lateral thickenings of the velar area in *Patella*. From the bottom of this pit a bilobed mass of cells is separated off, which differentiates itself into two lateral masses of nerve cells with fibres between them (*c.p*, Figs. 269, 270, and 271). The **pedal** and **visceral ganglia** arise as two pairs of thickenings of the ectoderm of the ventral surface, one pair being situated close behind the other (Fig. 269). In addition a pair of **pleural ganglia** make their appearance as a small pair of thickenings of the lateral ectoderm of the body, half-way between the rudiments of the cerebral and pedal ganglia; in later life they fuse with the cerebral ganglia (Fig. 274).

The **otocysts** arise as small spherical invaginations of ectoderm at the apex of the mantle-groove. They are situated in the region which is afterwards converted into the foot.

Towards the end of larval life the **foot** makes its appearance. It is defined by two transverse furrows, an anterior and a posterior, the latter cutting in deeply between the pedal and the visceral ganglia and separating them from one another. On the posterior aspect of the foot a deep invagination occurs which is lined by columnar cells. This is the rudiment of the **byssus gland**, which secretes the cords of horny material by means of which the adult *Dreissensia* anchors itself. The forepart of the foot grows into a finger-like process covered with minute cilia, and the primitive kidney disappears.

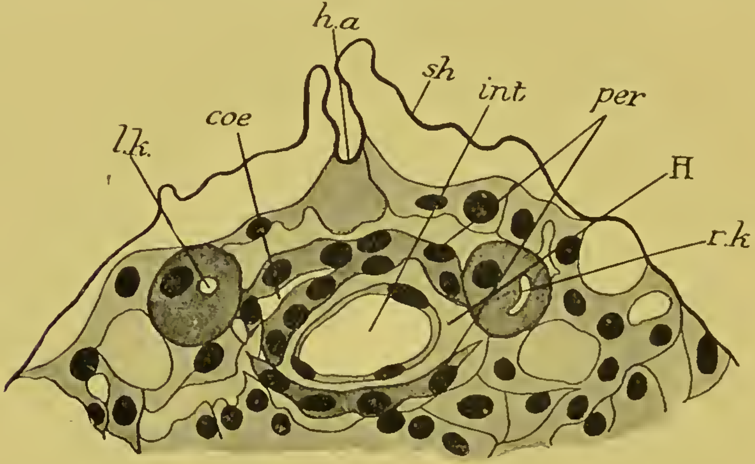

FIG. 272.—Transverse section through the dorsal region of old Veliger of *Dreissensia polymorpha* in order to show the differentiation of the pericardium and the kidneys. (After Meisenheimer.)

coe, first rudiment of pericardial cavity; *H*, heart; *h.a*, hinge area of shell; *int*, intestine; *l.k*, left kidney; *per*, ring of cells which gives rise to pericardium; *r.k*, right kidney; *sh*, shell.

The intestine becomes bent into a slight loop; it runs upwards from the stomach and bends downwards and forwards to reach the anus.

The **posterior adductor** muscle is formed by a modification of some of the spindle-shaped cells of the mesenchyme, and so also is the **retractor of the foot**. This retractor muscle is a mass of fibres which project downwards from the posterior part of the mid-dorsal region surrounding the end of the intestine and extend into the hinder region of the foot.

The first rudiments of the **gills** appear as a row of short, knob-like, ciliated protrusions from the roof of the mantle-groove, on each side and parallel with the posterior surface of the foot (*br*, Fig. 271). The **coelomic rudiment** becomes divided into a rounded mass of cells on each side of the intestine, which are the rudiments of one **kidney**, and into an arch of cells above the gut connecting these two rudiments. In the very last stage before metamorphosis this arch becomes a ring of cells surrounding the intestine (Fig. 272).

The rudiment of the cerebral ganglia becomes detached from the cerebral pit, and the cells forming the apical plate degenerate, cast off their cilia, and disappear (Fig. 273). The loop of the intestine becomes very long, so as to extend upwards parallel to the left side of the stomach.

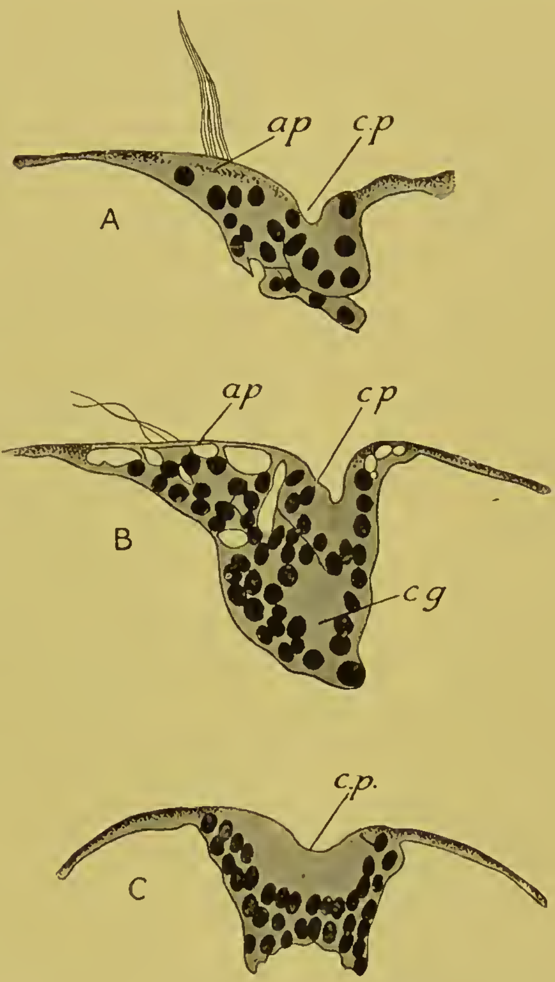

FIG. 273.—Sections through the cerebral pit of Veliger larvae of *Dreissensia polymorpha.* (After Meisenheimer.)

A, longitudinal section through the pit of a larva, in which the apical plate is fully developed. B, longitudinal section through the pit in a larva in which apical plate is degenerating. C, transverse section through the anterior part of the pit in a Veliger to show its bilobed character. *a.p*, apical plate; *c.g*, rudiment of cerebral ganglion; *c.p*, cerebral pit.

The **metamorphosis** of the Veliger into the mussel takes place with startling rapidity; it is as sudden as the change which converts the late free-swimming larva of *Polygordius* into the adult worm. As in that case, so here, the velar cells die and are cast off, the larval muscles break up and disappear, the whole anterior region in which the mouth was situated shrinks, and the cells forming the mouth cone degenerate and disappear. The result of this change is to bring the mouth and the anterior adductor closer to one another, and thus to swing the foot round so that its apex points forwards instead of downwards. The rows of gill papillae are swung round from a vertical to a horizontal position, and the loop of the intestine is straightened out.

After metamorphosis the **labial palps,** so characteristic of the adult mussel, are formed in an amazing manner. The cerebral pit, from which the rudiments of the cerebral ganglia have been separated, flattens out and forms two lateral bands of ciliated epithelium above and at the sides of the mouth. These develop into the upper and outer labial palps, while downgrowths from their inner ends give rise to the inner and lower labial palps (Fig. 276).

The shell begins to alter its larval shape by a preponderant growth of its posterior and lower angle, and by a certain amount of growth of its anterior angle also. The gill papillae grow into long filaments, which become locked together by the longer cilia on their lateral faces. The development of an additional outer row of gill filaments, and the bending up of the ends of the filaments to form the reflected portions of the gill lamellae, take place later in life (Fig. 275).

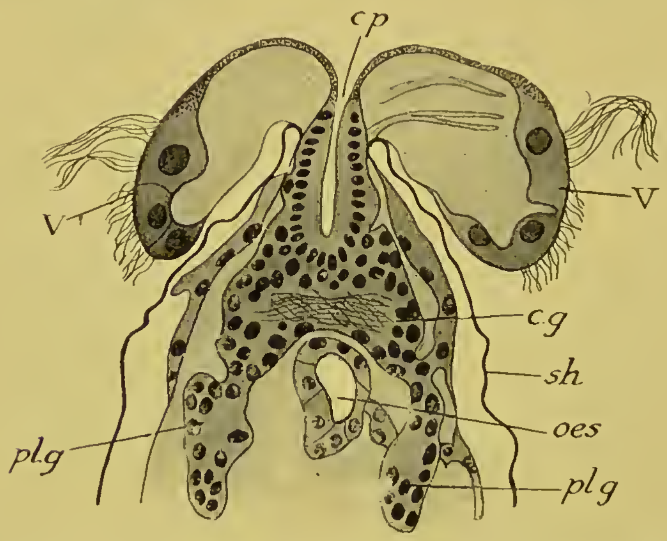

FIG. 274.—Horizontal section through the anterior portion of an old Veliger of *Dreissensia polymorpha* in order to show the differentiation of the cerebral ganglia from the cerebral pit. (After Meisenheimer.)

c.g, cerebral ganglion; *c.p*, cerebral pit; *oes*, oesophagus; *pl.g*, rudiment of pleural ganglia; *sh*, shell; V, velum.

The rudiments of the **kidneys** acquire cavities. The ring of cells surrounding the intestine becomes double and the two layers separate from one another, the cavity between them being the **pericardium,** *i.e.* the coelom. The cavity of the **heart** is the space between the inner of these layers and the intestine. The kidney becomes U-shaped, and the inner limb of the U on either side (which will form the **ureter**) coalesces with the corresponding part of the other kidney so as to form a transverse space beneath the intestine. Into this space opens, on either side, a diverticulum of the mantle-cavity, and in this way the **external opening** is formed. The greater part of the ureter

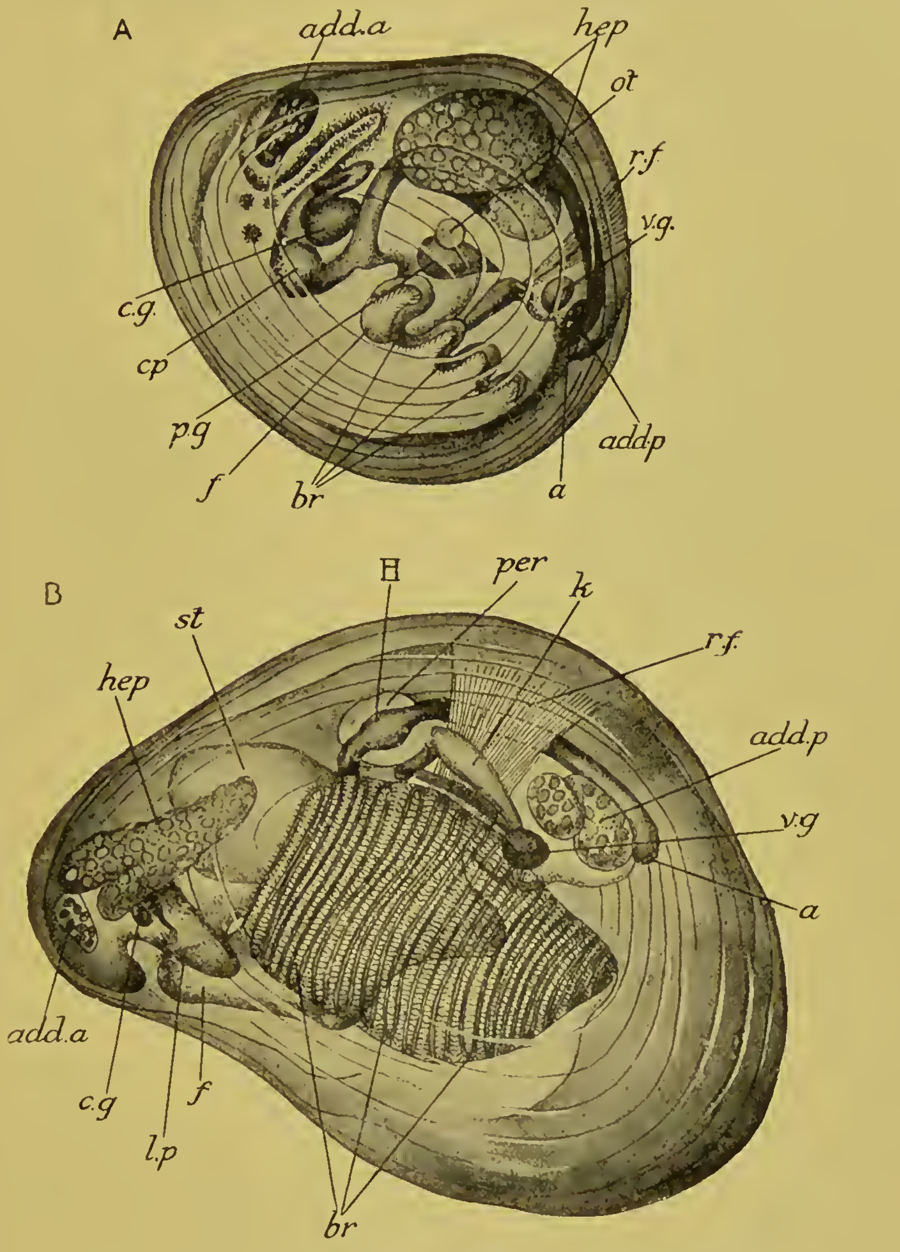

FIG. 275.—Side views of two young specimens of *Dreissensia polymorpha* after the metamorphosis has taken place. (After Meisenheimer.)

A, young specimen in which the shell retains the shape it possessed in the veliger. B, older specimen in which the shell is beginning to assume its adult proportions. *a*, anus; *add.a*, anterior adductor muscle; *add.p*, posterior adductor muscle; *br*, rudiments of gills; *c.g*, cerebral ganglion; *c.p*, cerebral pit; *f*, foot; *H*, heart; *hep*, liver; *k*, kidney; *l.p*, labial palp; *ot*, otocyst; *per*, pericardium; *p.g*, pedal ganglion; *r.f*, retractor of foot; *st*, stomach; *v.g*, visceral ganglion.

is therefore not ectodermal but coelomic in origin. The limb of the kidney which was originally the outer limb, bends inwards and fuses with the lower part of the pericardium, and here the **reno-pericardial canal** is formed (Fig. 277).

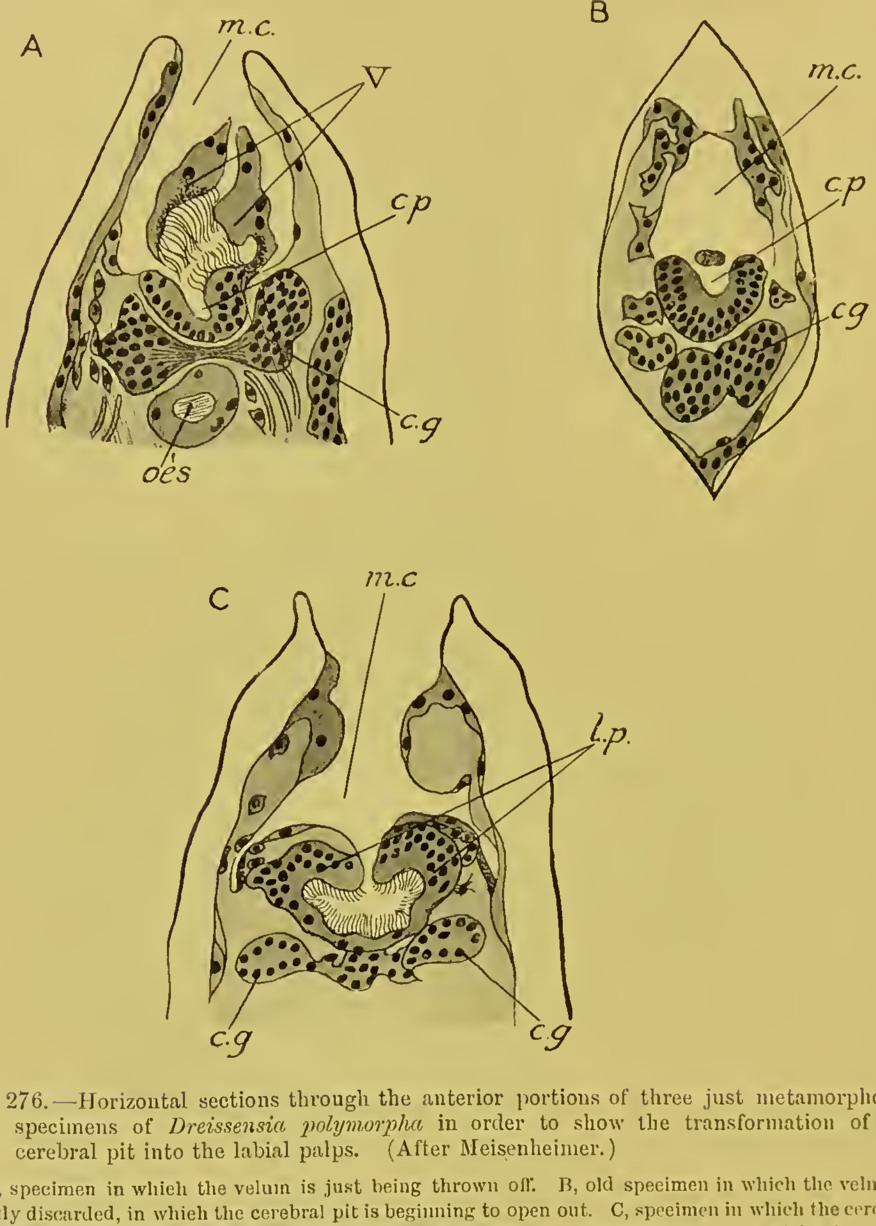

Fig. 276.—Horizontal sections through the anterior portions of three just metamorphosed specimens of *Dreissensia polymorpha* in order to show the transformation of the cerebral pit into the labial palps. (After Meisenheimer.)

A, specimen in which the velum is just being thrown off. B, old specimen in which the velum is already discarded, in which the cerebral pit is beginning to open out. C, specimen in which the cerebral pit has given rise to two ciliated lobes. *c.g*, cerebral ganglion; *c.p*, cerebral pit; *l.p*, labial palps; *m.c.* mantle-cavity; *oes*, oesophagus; V, discarded velar cells.

The **genital organ** arises from a median ventral strip of the pericardial wall, in front, just between the openings of the reno-pericardial canals. It consists of peculiar large cells with pale nuclei. These cells multiply, become detached from the pericardial wall, and

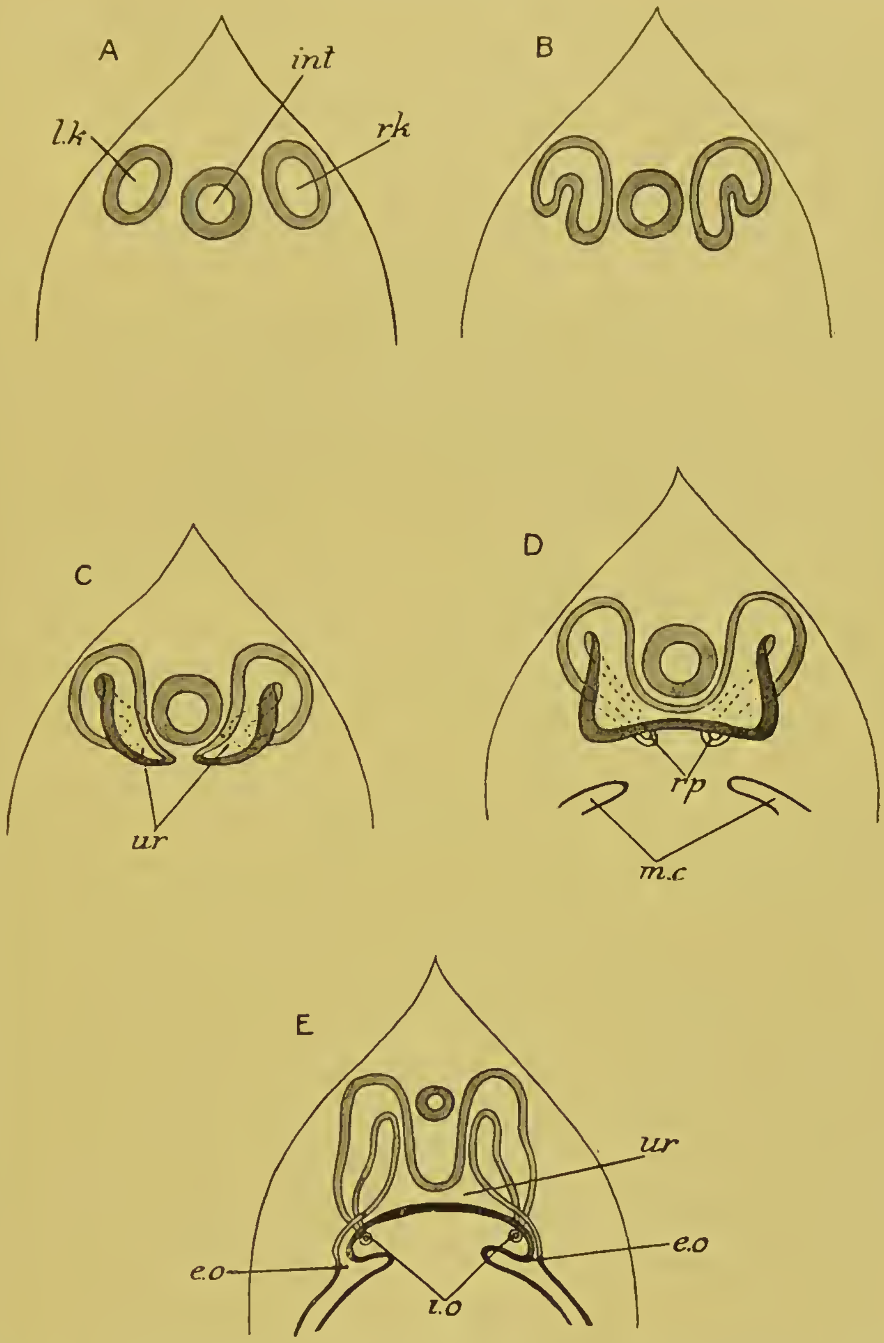

FIG. 277.—Diagrammatic transverse sections of young specimens of *Dreissensia polymorpha* in order to illustrate the development of the kidney. (After Meisenheimer.)

A, the kidney a round sac. B, the kidney assumes a U-shape—the outer limb develops into the glandular portion, the inner limb into the ureter. C, the outer limb becomes bent inwards toward the middle line. D, the two inner limbs—the rudiments of the ureters fuse in the middle line. E, the internal and external openings are formed. *e.o*, external opening of the kidney; *int*, intestine; *i.o*, internal opening of the kidney; *l.k*, left kidney; *m.c*, mantle-cavity; *r.k*, right kidney; *r.p*, reno-pericardial canal; *ur*, ureter.

divide into masses which, in the latest stages examined by Meisenheimer, are found beneath the pericardium and lying not far from the lateral ectoderm on each side. The formation of the genital ducts was not observed by him (Fig. 278).

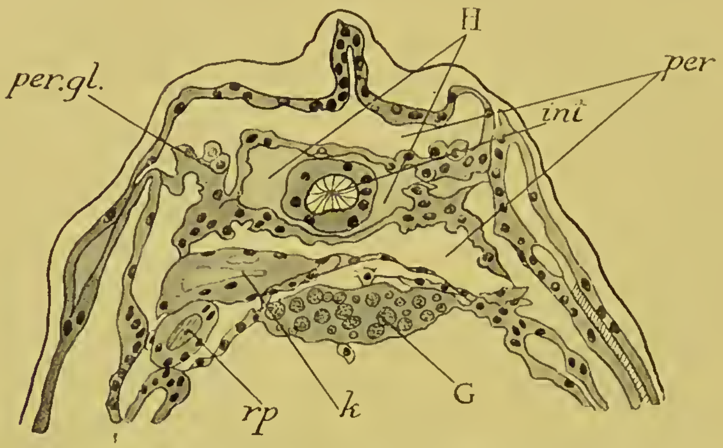

FIG. 278.—Transverse section through a young *Dreissensia polymorpha* in order to show the origin of the genital organs. (After Meisenheimer.)

G, median mass of genital cells forming a thickening in the floor of the pericardium ; *H*, heart ; *int*, intestine ; *k*, kidney ; *per.gl*, pericardial gland ; *per*, pericardium ; *r.p*, reno-pericardial canal.

Our account of the development of *Dreissensia* is now complete. We must pause, however, and glance at what is known of the development of other Pelecypoda before considering the development of the highest Mollusca.

OTHER PELECYPODA

The development of no other Pelecypod has been worked out with anything like the same completeness as *Dreissensia*. What we know of other life-histories are mainly bits and scraps. From the accounts, however, given by Horst (1882) of the development of *Ostrea*, by Drew (1906) of that of *Pecten*, by Hatschek (1885) of that of *Teredo*, by Sigerfoos (1895) of that of *Pholas*, and by Löven (1848) of that of *Cardium*, we can only conclude that the development of all these forms is practically identical with that of *Dreissensia*. The figures given of the veliger larvae are so similar that one would almost be driven to conclude that there is a veliger larva of definite type common to all marine Pelecypoda, and that the differentiation of the various genera from one another takes place during post-larval life. Indeed, the researches of Stafford (1910) on the veliger larvae found in the lagoons of Prince Edward Island, and off the New Brunswick coast, have gone far to bear out this conclusion. Amongst other things he has shown that the late veliger larva of *Ostrea virginiana* possesses a well-marked foot which is used for locomotion in the early post-larval stages, before the definitely fixed life of the adult is assumed (Fig. 279).

The only point which requires some comment is the description given by the earlier workers of the segmentation stages. Thus Horst (1882) and Hatschek (1883) both describe the endoderm as represented by one huge macromere, which buds off the micromeres which give rise to the ectoderm; instead of there being, as in all other Mollusca, four macromeres. There is strong ground for believing that this is a misinterpretation, and that in all cases four macromeres are really formed, but that, as is the case with *Dreissensia*, one is much larger than the rest.

To the statement that the development of Pelecypoda, up to the veliger stage, pursues a uniform course in all genera, two marked exceptions must be made. The first of these concerns the group of the Protobranchiata, including the genera *Nucula*, *Leda*, *Yoldia*, etc., whose development has been studied by Drew (1899, 1901).

In this group the velum acquires enormous dimensions, and consists of circles of large vacuolated cells placed one above the other, forming a barrel-shaped structure. The first and last circles bear numerous small cilia all over their surface, and the central three circles have each a narrow band of long cilia (Fig. 280). A sagittal section through this extraordinary structure reveals inside it a saddle-shaped shell gland, a long narrow stomodaeum leading up to a stomach, and a cerebral ganglion arising in *Yoldia* as a pit in front of the apical plate (Fig. 281). The foot appears later, and when the metamorphosis occurs and the velar cells are cast away, the cilia covering the foot are sufficiently powerful to enable the animal to glide over the mud in which it lives before any burrowing movements are carried out. The general plan of the development is therefore the same as in *Dreissensia*.

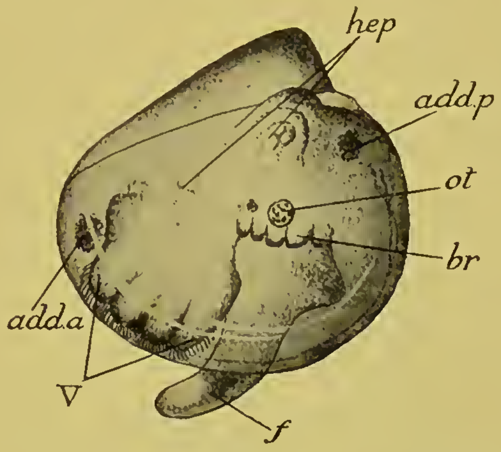

Fig. 279.—The late Veliger larva of *Ostrea virginiana*, viewed from the side. (After Stafford.)

add.a, anterior adductor muscle; *add.p*, posterior adductor muscle; *br*, rudiments of gills; *f*, foot; *hep*, lobes of liver; *ot*, otocyst; V, retracted velum.

At the other pole of variation are freshwater forms like *Cyclas*, *Pisidium*, etc., and the family of the Unionidae, where the early stages of development are passed between the lamellae of the gill of the mother, and where is therefore no free-swimming stage and neither prototroch nor velum is developed.

In Ziegler's account of the development of *Cyclas* (1885) it is stated that there is only one large macromere from which all the micromeres are budded off. As we pointed out above, this is probably a misinterpretation of the early stages of development. *Cyclas* is further remarkable for the fact that the coelom makes its appearance as two vesicles situated at the sides of the intestine. These vesicles become constricted into dorsal and ventral halves, but they meet one another both above and below the intestine, and hence, in another way, the same end results as that attained in *Dreissensia*. The space between dorsal and ventral halves forms the **auricle** of the heart, that between the vesicles and the intestine forms the **ventricle** (Fig. 282).

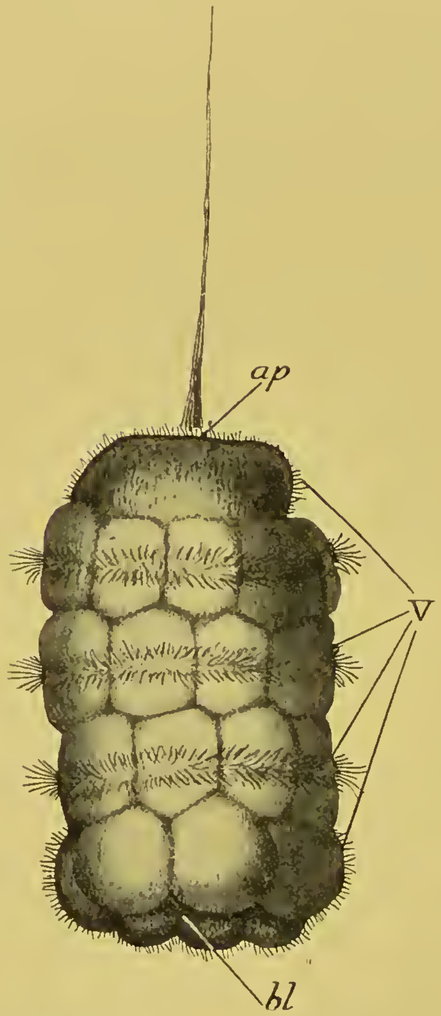

Fig. 280.—The Veliger larva of *Yoldia limatula*, about three days old. (After Drew.)

a.p, apical plate ; *bl*, position of blastopore ; V, velum.

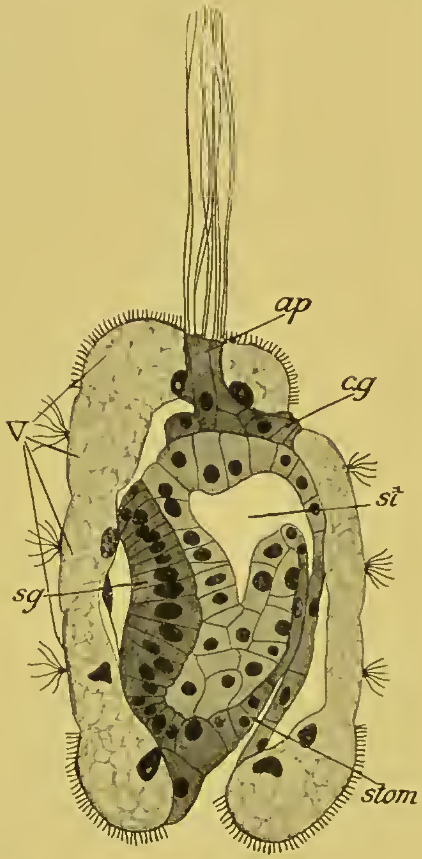

Fig. 281.—Longitudinal sagittal section of the Veliger larva of *Yoldia limatula*. three days old. (After Drew.)

a.p, apical plate ; *c.g*, rudiment of cerebral ganglia (representative of cerebral pit) ; *s.g*, shell gland ; *st*, stomach ; *stom*, stomodaeum ; V, velar cells.

The Unionidae give rise to an extraordinary larva, known as a **Glochidium** ; it is devoid of mouth, velum, and foot, but provided with a bivalve shell, and the lower borders of the valves are each provided with a sharp, inturned tooth. This larva is capable of only a few spasmodic flappings of the valves of the shell, which propel it through the water for a short distance. It is ejected from the gills by the parent when a freshwater fish happens to pass in the vicinity, and a successful larva contrives to fix itself on the gills or fins of the passing fish by grasping them by means of the valves of the shell. The bite of the valves stimulates growth of the soft vascular gill, so that the Glochidium is soon enclosed in a cyst in which it completes its development, and from which it emerges only when it has attained the adult condition (Fig. 283).

The best account of the early development of Unionidae has been given by Lillie (1895), and the most recent worker at the post-larval development is Harms (1909). Lillie's account is interesting in making it quite clear that, in spite of its aberrant appearance, the development of the embryo of *Unio* conforms to the scheme given for *Dreissensia*. There is, it is true, no prototroch, and the first quartette of micromeres divide only once or twice and form the "head vesicle." On the other hand, as in *Dreissensia*, the first somatoblast, 2d (X), is enormous, and it divides in exactly the same way as in *Dreissensia*. The group of small cells along its lower edge (x_1-x_5) give rise to what Lillie calls the **ventral plate**, a thickened region of the ectoderm from which the foot is formed in post-larval life. There is a primary mesoderm cell, 4d, which divides into right and left halves, from each of which a packet of cells is formed, parts of which break up into mesenchyme. The shell gland is enormous and the endodermic rudiment very small. As the shell gland becomes everted the

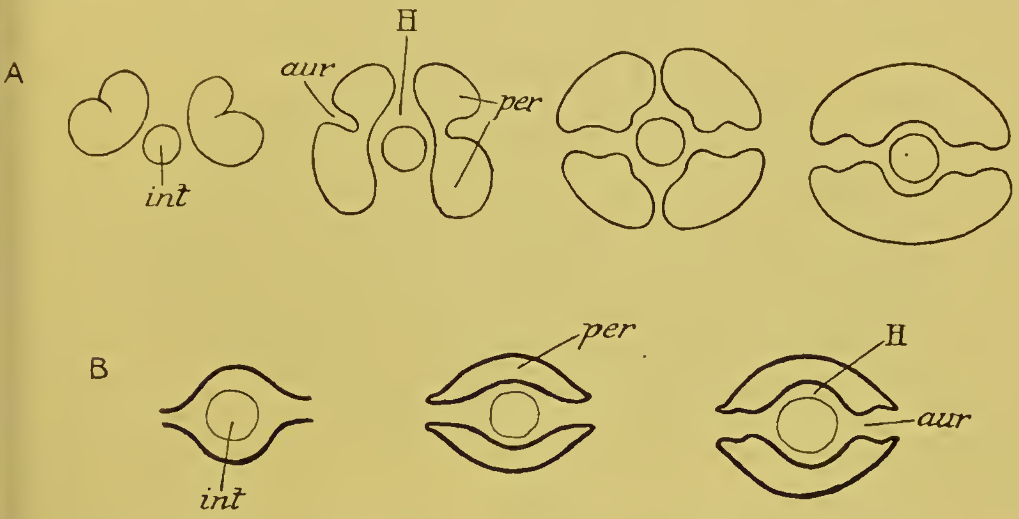

FIG. 282.—Diagrams illustrating the development of the pericardium in *Cyclas* and *Dreissensia*. (After Meisenheimer.)

A, development of pericardium in *Cyclas*. B, development of pericardium in *Dreissensia*. *aur*, space which forms the auricle of the heart; *H*, heart; *int*, intestine; *per*, pericardium.

endodermic rudiment is invaginated and the blastopore closes, but the stomodaeum remains as a thickened plate of ectoderm during the Glochidium stage. Rudiments of three pairs of ganglia are sometimes present (*Anodon*), and sometimes not (*Unio*). On each side the mantle-lobe bears three sense cells with long sense hairs. There is a powerful adductor muscle connecting the valves of the shell.

In the post-larval life the most marked feature is the modification of the cells forming the larval mantle. These cells develop into huge vacuolated columnar structures which actually absorb and digest fragments of the blood cells and other tissues of the host which enter the mantle-cavity of the parasite. The cells destined to form the **adult mantle** arise from the apices of the two mantle-grooves and spread downwards, displacing the larval mantle cells, which are eventually shed (Fig. 284). We can only compare these rudiments of the adult mantle to the imaginal discs of insects. The single **larval adductor muscle** disappears and is replaced by the **adult adductor,** of which sometimes the anterior and sometimes the posterior is the first to be formed. The foot, gills, and otocysts arise exactly as in *Dreissensia.* The coelom arises as two little packets of cells, one on each side, closely attached to the ectoderm, from which Harms supposes them to have been derived; but this view, for reasons given above, we cannot accept. The later development of these rudiments is exactly the same as in *Dreissensia*; the main mass on each side

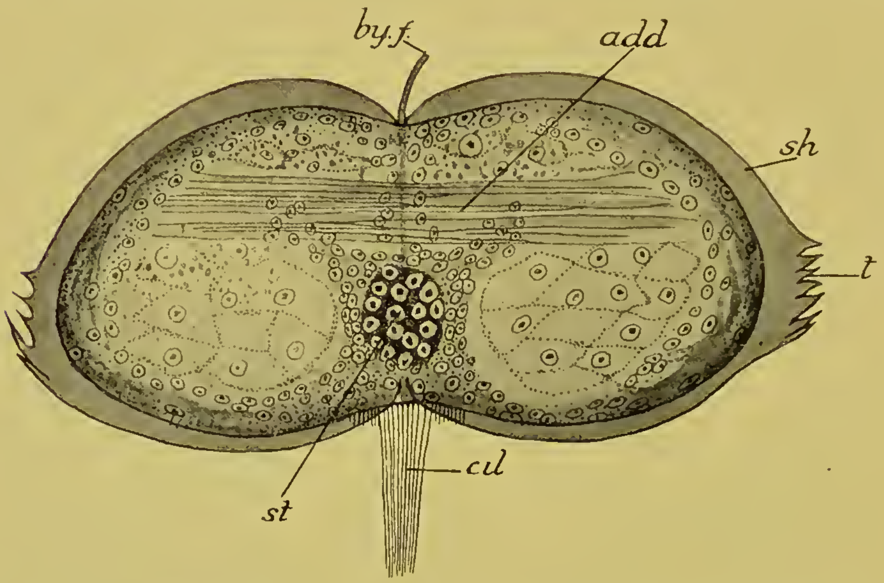

FIG. 283.—Glochidium—larva of *Margaritana* with widely opened valves, viewed as a transparent object from the dorsal surface. (After Harms.)

add, adductor muscle; *by.f*, byssus thread; *cil*, ciliated patch on the ventral surface; *sh*, larval shell; *st*, stomach; *t*, teeth of shell.

hollows out to form the kidney, whilst a band of cells grows out from each and forms a ring round the gut, splits into two layers, and forms the pericardium.

The accounts of Harms and Lillie leave no doubt in the mind that in the embryo of the Unionidae we are merely dealing with an ordinary Pelecypod veliger modified for a parasitic existence.

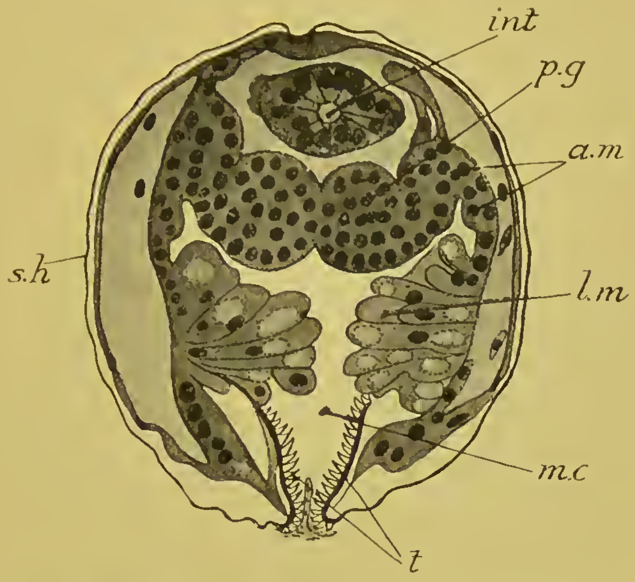

Fig. 284.—Transverse section of a Glochidium larva of Unio which is already fixed in the tissues of its post. (After Harm.)

a.m, cells which will form the adult mantle; *int*, intestine; *l.m*, vacuolated cells of larval mantle, which absorb food material from the host; *m.c*, mantle-cavity; *p.g*, rudiments of pedal ganglia; *t*, teeth of larval shell.

CEPHALOPODA—*Loligo, Sepia*

We must now turn to the study of the embryology of the highest Mollusca, the Cephalopoda (lit. head-footed), so called because the fore part of the foot has grown into a frill surrounding the head. Two genera are represented by common species, both on the English coasts and in the Mediterranean; these are *Sepia officinalis* and *Loligo vulgaris*. So far as is known, the development of both pursues a practically identical course. We shall select *Loligo* as a type for special study, because its development has been more completely worked out, and because species of this genus are common on the American coast; but we shall not hesitate to fill up lacunae in our knowledge of the development of *Loligo* by the description of corresponding stages from the development of *Sepia*, when these are better known.

The eggs of both genera, like all Cephalopod eggs so far described, contain an abundance of yolk, the cytoplasm being mainly restricted to a small disc at the animal pole of the egg, in which the nucleus is situated. The Cephalopod egg is, in fact, the *beau ideal* of a telolecithal egg. The egg of *Sepia* is nearly spherical, about the size of a pea; it is enclosed in a tough black chorion of the consistence of india-rubber, difficult to remove. The egg of *Loligo*, on the contrary, is about the size of an apple pip, and is of an elongated oval shape. Many eggs are laid together immersed in a somewhat tough jelly, which can be partly dissolved by exposure to the action of Eau de Javelle for fifteen minutes.

For the study of segmentation stages surface views are essential, and for this purpose the most superficial layer, including all the cytoplasm, is removed from the animal pole of the egg by means of a sharp knife, and the skin thus obtained is spread out flat. Even when sections are desired it is inadvisable to endeavour to cut through the whole mass of yolk; only a small part of the upper half of the egg should be removed and cut into sections.

The segmentation of *Sepia* has been carefully described by Vialleton (1888). Minchin began a renewed study of the subject and made a series of exquisite preparations from eggs preserved in Hermann's fluid, which, however, he did not describe; but these have been described by Koeppern (1909), and his results confirm in every detail those of Vialleton, whose account we follow here. No such exhaustive account of the early stages of development of *Loligo* is available, but, from what is known of it, it agrees with that of *Sepia* in every particular.

According to Vialleton, then, the cleavage of the egg of *Sepia* is **meroblastic**, that is to say, it is only the protoplasmic end of the egg which is divided by the cleavage furrows, the yolk being quite unaffected. When the nucleus has divided into four, and two cleavage furrows at right angles have been formed, we have obviously a stage which corresponds to the stage of division of other Molluscan eggs into four macromeres.

The next cleavage furrow is a circumferential one and cuts off four inner cells, termed **blastomeres** by Vialleton, from four outer large cells whose lower ends fade into the yolk, which he terms **blastocones**. This stage corresponds roughly to the stage of the formation of the first quartette of micromeres in other Molluscan eggs, but no one has attempted to work out the cell-lineage in a Cephalopod egg, and it will become obvious that many more cleavages are necessary to separate one from another the specific materials of the germinal layers than is the case with the vastly smaller eggs of other Mollusca. For example, in the 32-cell stage of *Sepia* (Fig. 285), if the blastocones be regarded as corresponding to the macromeres, it is obvious that there must be at least 20 macromeres and only 12 micromeres, whereas in a normal Molluscan egg there would be 4 macromeres and 28 micromeres in the 32-cell stage.

By further radial cleavage furrows the number of blastocones is greatly increased, and by new circumferential furrows the inner portions of these are continually cut off as new blastomeres, whilst the already existing blastomeres undergo division, and in this way a one-layered sheet of cells, or "**blastoderm,**" becomes spread over the upper surface of the egg.

When the blastoderm has extended so as to form a sheet of cells covering about one-eighth of the surface of the egg, the formation of new blastomeres from the blastocones ceases: these latter now appear as a series of narrow, spoke-like pillars radiating from the blastoderm as a hub. The basal portions of these spokes become narrower and narrower, as their nuclei wander farther and farther away from the blastoderm, till they become mere threads. Finally the portion with the nucleus separates altogether from the blastoderm and begins to divide, and in this way a membrane is formed consisting of a single layer of extremely flattened cells, which rapidly extends all over the yolk; it extends also underneath the blastoderm so as to cover the yolk there also. This sheet of cells is termed the **yolk-membrane**; it has been rightly compared to a portion of the endoderm. Its cells exercise some digestive influence on the yolk and gradually change it into a form that can be used for the nourishment of the embryo. But no part of this yolk-membrane is incorporated in the permanent epithelium of the mid-gut. The cells from which the mid-gut epithelium will be formed, arise later by divisions of the most

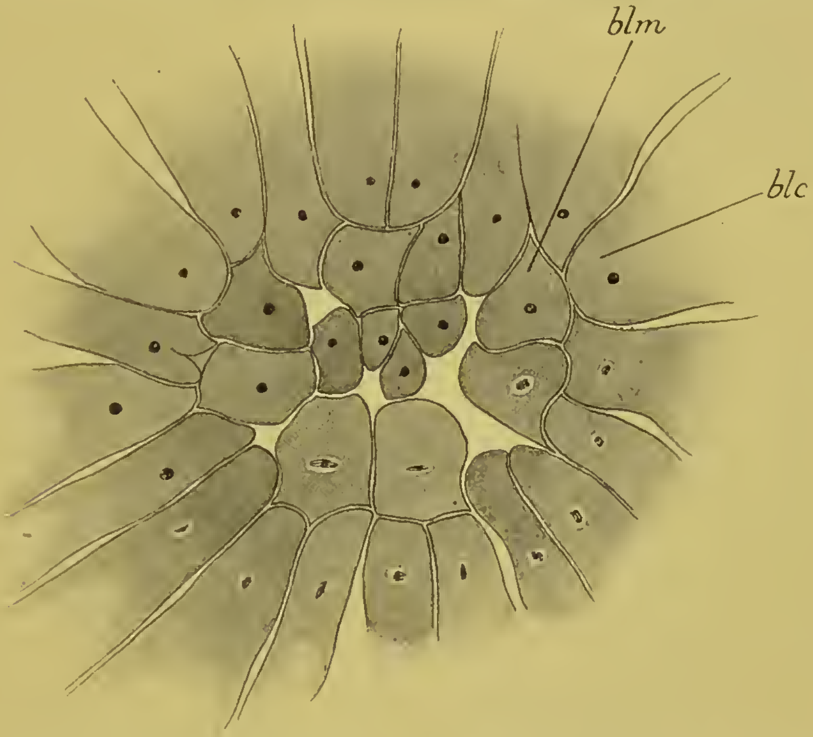

FIG. 285.—Segmenting egg (32-cell stage) of *Sepia officinalis* viewed from the animal pole. (After Koeppern, from Minchin's preparations.)
blc, blastocone; *blm*, blastomere.

peripheral blastomeres of the blastoderm, at right angles to the surface. These divisions take place along what afterwards are seen to be the posterior and lateral edges of the blastoderm. In this way an incomplete ring of what are called "**lower layer cells**" is formed, which former observers have termed mesoderm but which we may term **mesendoderm.** Lankester (1875) maintains that there is a "**primitive streak**" in the segmenting eggs of Cephalopoda, by which he means a restricted area of the surface over which the proliferation of cells which gives rise to the mesendoderm takes place. *A priori* this is very likely, although so far this observation has not been confirmed. This mesendoderm grows inwards under the blastoderm to a considerable extent, but does not invade, except in the form of a few loose scattered cells, its front edge or its centre.

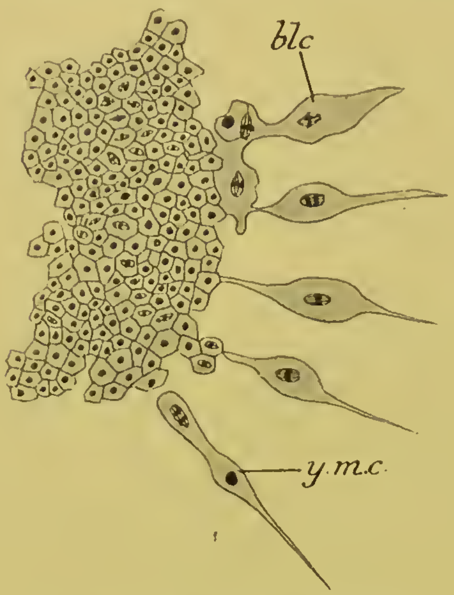

FIG. 286.—A portion of the margin of the blastoderm of the egg of *Sepia officinalis* at the conclusion of the process of segmentation; to show the transformation of the blastocones into cells of the yolk-membrane. (After Koeppern, from Minchin's preparations.)

blc, blastocones; *y.m.c*, yolk-membrane cells.

The formation of definite organs in the embryo of *Loligo* has been worked out in comparatively recent times by Korschelt (1892) and by Faussek (1900). These workers found that Hermann's fluid, and similar mixtures containing osmium tetroxide, render the tissues too brittle for section-cutting although giving excellent histological detail. They therefore employed picro-sulphuric acid and Perenyi's fluid, which gave excellent preservation of form, although the histological detail is not so perfect as that obtained by the use of Hermann's fluid.

The first organ to make its appearance is the **shell gland.** This appears as a heart-shaped area of columnar ectoderm in the mid-dorsal line. This thickening in fact occupies the apex of the egg; in front of it is what we may call the anterior slope, behind it the posterior slope. Soon an invagination appears in the centre of this thickening; but, as Lankester long ago pointed out (1875), this does not correspond to the invagination of the shell gland which we encounter in other Molluscan embryos. That invagination precedes the condition of the gland when it is a saddle-shaped area, and lasts only a short time, but the invagination in the shell gland area of a Cephalopod lasts throughout life; it constitutes, in fact, the **shell-sac** which encloses the **shell** (a horny pen in *Loligo*, but a complex calcareous structure in *Sepia*). From a comparison with the primitive genus *Nautilus*, we know that this covering in of the shell by mantle flaps is a secondary phenomenon which was not present in the earlier Cephalopoda.

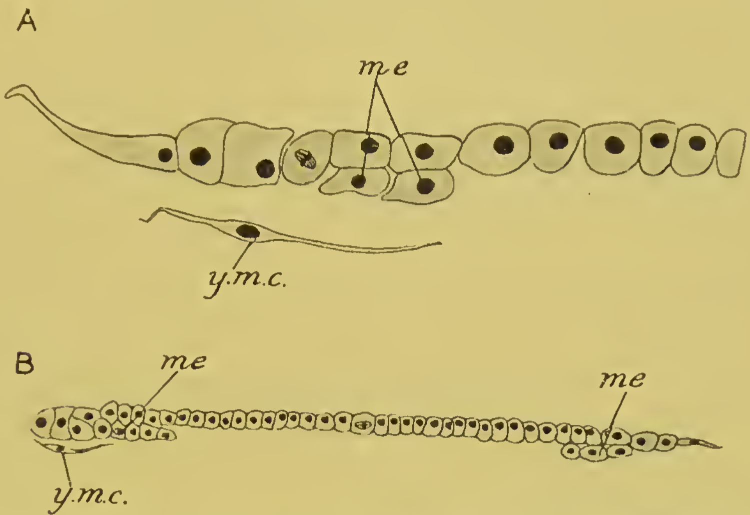

FIG. 287.—Two sections through the edge of the blastoderm of *Sepia officinalis* in different stages of development; to illustrate the development of the lower layer cells. (After Koeppern, from Minchin's preparations.)

A, Younger stage. B, Older stage. A is more highly magnified than B. *me*, mesoderm, *i.e.* lower layer cells; *y.m.c*, yolk-membrane cell.

The **eyes** now appear as shallow cups on the sides of lateral protrusions of the body, which may be termed **eye-stalks**. The edges of the Cephalopod shell gland constitute, as in other Mollusca, the rudiment of the mantle, and underneath them appears a groove, deepest behind, which is the rudiment of the **mantle-cavity**. Just within this groove two buds appear to the right and left of the middle line; these are the rudiments of the **gills**: whilst below them appear two pairs of ridges converging towards the ventral surface and

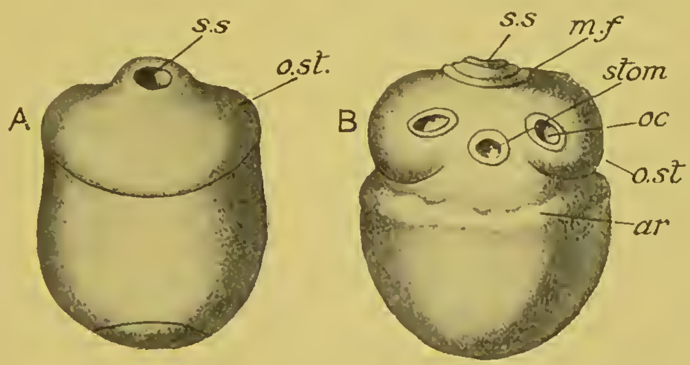

FIG. 288.—Two early embryos of *Loligo vulgaris*. (After Korschelt.)

A, An embryo seen from the posterior side. B, A slightly older embryo seen from the anterior side. *ar*, rudiments of arms; *m.f*, mantle-fold; *o.c*, optic cup; *o.st*, optic stalk; *s.s*, shell-sac; *stom*, stomodaeum.

constituting the rudiment of the **hind foot** or **funnel.** These are termed the **anterior** and **posterior funnel folds** respectively. The rudiments of the **fore foot,** the arms, appear as a series of thickenings on the lower edge of the blastoderm (Figs. 288, 289).

Whilst these changes have been going on, the first trace of the **mid-gut** makes its appearance as a thickening of the "mesendoderm" on the posterior slope of the egg; whilst the stomodaeum appears as an invagination in the middle line of the anterior slope of the embryo.

In the endodermal thickening a cavity is formed by the separation of its constituent cells from each other. This cavity is at first closed internally merely by the yolk-membrane; but it soon independently acquires its own internal wall, whilst externally it raises the ectoderm into a slight papilla, the **anal papilla,** at the apex of which the **anus** appears a little later. There is no proctodaeum. Before the anus is perforated the incipient mid-gut gives off a diverticulum which is the rudiment of the **ink gland** (Fig. 290, B).

The stomodaeum is at first a shallow cup, but it soon extends up towards the mid-dorsal line and past it, where, in a much later period of development, it fuses with and opens into the mid-gut. The **radula sac** appears as a ventral outgrowth of the stomodaeum, and a still more ventral outgrowth is the rudiment of the **salivary gland.** The **otocysts** now arise as open pits near the junction of the anterior and posterior funnel folds. These two pairs of folds, as already noted, are the rudiments of the funnel.

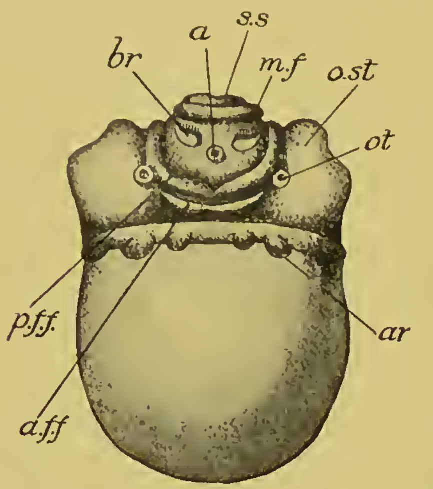

FIG. 289.—Embryo of *Loligo vulgaris* seen from the posterior side at the conclusion of the period of development, termed by Faussek Stage 1. (After Faussek.)

a, anus; *a.f.f*, anterior funnel fold; *ar*, rudiment of arms; *br*, gill rudiment; *m.f*, mantle fold; *p.f.f*, posterior funnel fold; *o.st*, eye-stalk; *ot*, otocyst; *s.s*, shell-sac.

In this stage the rudiments of the three principal pairs of **ganglia** arise. The **cerebral ganglia** appear as long streak-like ectodermic thickenings running below the eyes; the **pedal ganglia** as much shorter streaks parallel to the hindermost portions of these and above them, extending to the otocysts; and finally the **pleural ganglia** are represented by short vertical streaks extending up towards the mantle. The shape of the rudiments reminds one of the band-like condition of the ganglia found in *Nautilus* (Fig. 291), and we may add that the appearance of the funnel as two unconnected ridges also recalls its condition in *Nautilus.*

The first trace of the **genital cells** appears in this stage as a packet of large pale cells with pale nuclei, situated on the posterior aspect of the embryo between the rudiment of the gills (Fig. 297, A).

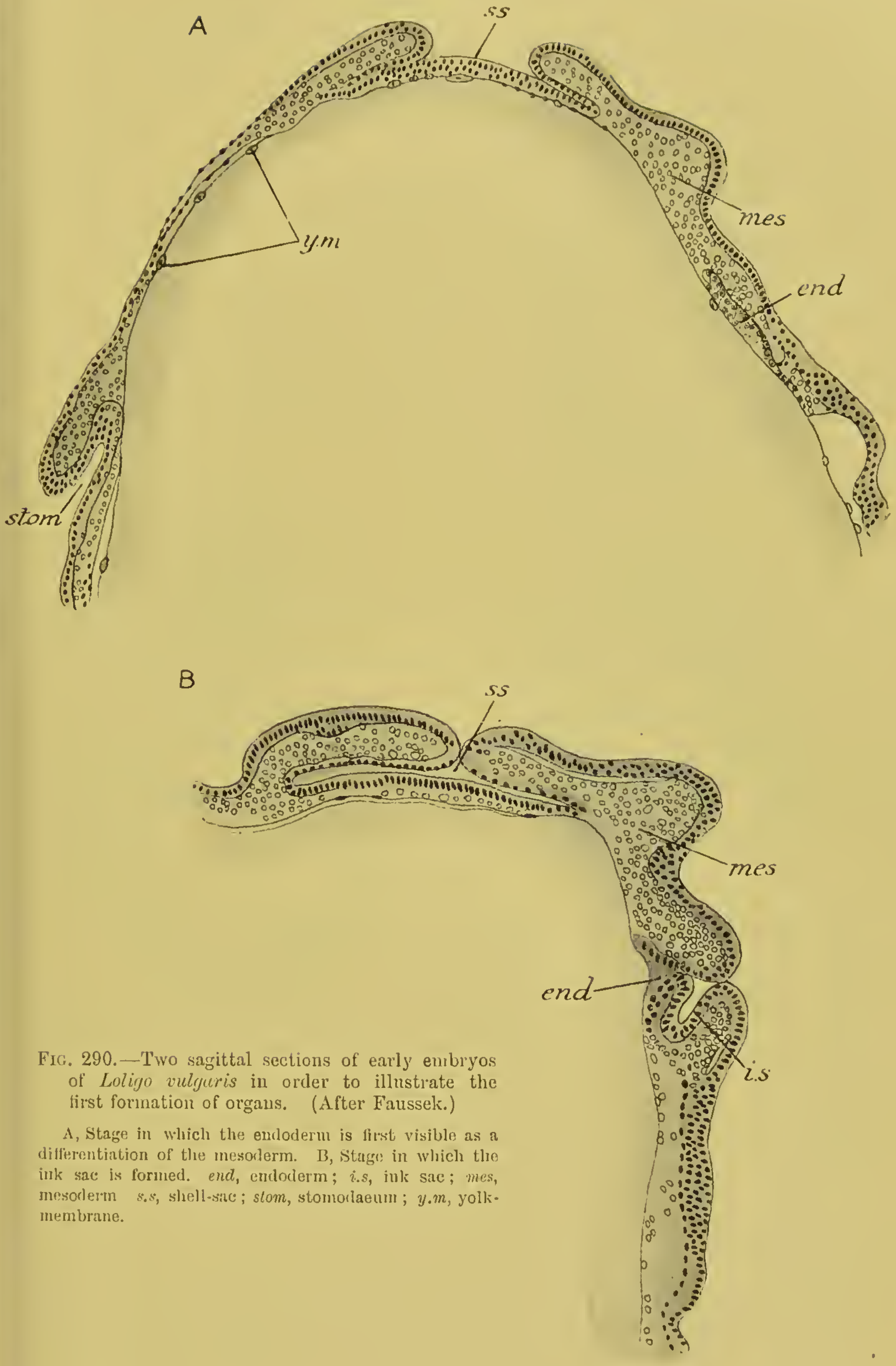

Fig. 290.—Two sagittal sections of early embryos of *Loligo vulgaris* in order to illustrate the first formation of organs. (After Faussek.)

A, Stage in which the endoderm is first visible as a differentiation of the mesoderm. B, Stage in which the ink sac is formed. *end*, endoderm; *i.s*, ink sac; *mes*, mesoderm *s.s*, shell-sac; *stom*, stomodaeum; *y.m*, yolk-membrane.

As development proceeds the upper portion of the egg, covered by the blastoderm, begins to be separated by a constriction from the lower part, which consists merely of yolk covered by the yolk-membrane, and so we are enabled to distinguish between "**embryo**" and **yolk-sac**. It must be remembered, however, that a considerable portion of the yolk is contained within the confines of the embryo; this is known as the **internal yolk-sac**, and is disconnected from the **external yolk-sac**, which appears as an appendage. The two are, of course, joined by a neck of communication.

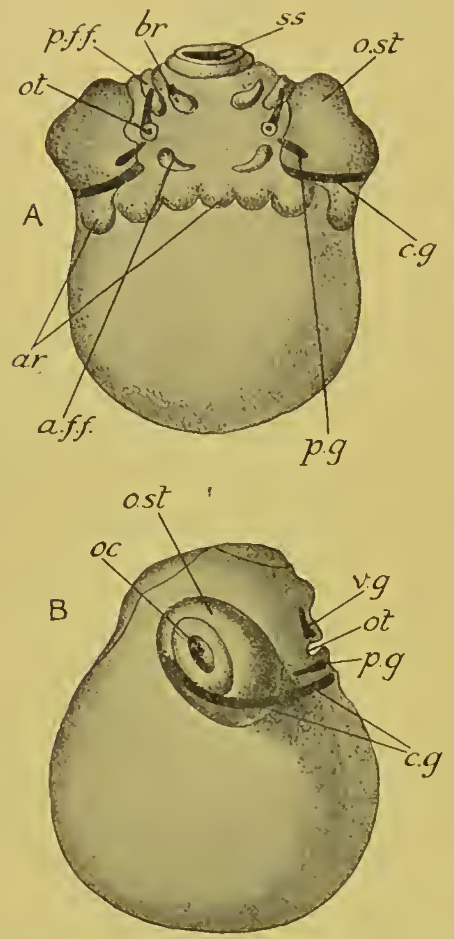

FIG. 291.—Embryo of *Loligo vulgaris* seen from the side and behind in order to illustrate the development of the ganglia. The embryo is younger than that represented in Fig. 289. (After Faussek.)

A, Embryo seen from behind. B, Embryo seen from the side. *a.f.f*, anterior funnel folds; *ar*, rudiments of arms; *br*, rudiment of gill; *c.g*, rudiment of cerebral ganglion; *oc*, eye-cup; *o.st*, eye-stalk; *ot*, otocyst; *p.f.f*, posterior funnel folds; *p.g*, rudiment of pedal ganglion; *s.s*, shell-sac; *v.g*, visceral ganglion.

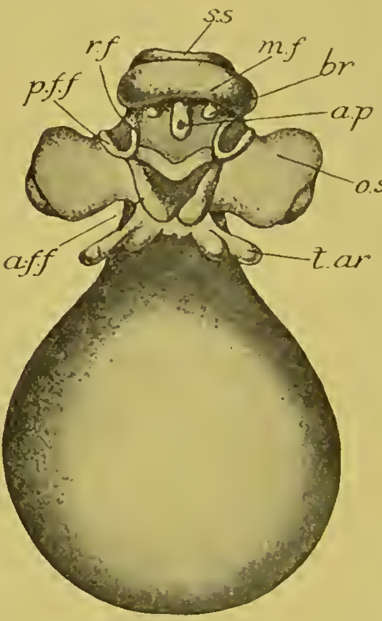

FIG. 292.—Embryo of *Loligo vulgaris* in the stage termed by Faussek Stage 2, when the embryo begins to be grooved off from the yolk-sac; posterior view. (After Korschelt.)

Letters as in previous figure. In addition, *a.p*, anal papilla; *r.f*, retractor muscle of funnel; *t.ar*, tentacular arms.

When the grooving off of the external yolk-sac has become distinct, many other changes take place. The eye-stalks grow in length, while the eye-pits become closed, and the inner segment of the **lens** is developed as a secretion from the anterior wall of each pit. The otocysts also become closed and sink into the ridges which are the rudiments of the funnel. The **retractor muscles** of the funnel appear as two ridges which stretch from the middle of the funnel, where anterior and posterior funnel folds have united, upwards to the edge of the mantle. The mantle edge extends over the rudiments of the gills, and the mantle cavity deepens; whilst the shell-sac closes, and the central region of the mantle becomes arched up so as to constitute a **visceral hump**. The rudiments of the arms lengthen; those of the **tentacular arms** exceed the others in length. The rudiments of the ganglia become constricted off from the thickenings of the ectoderm; that part of the thickenings giving rise to the cerebral ganglia which lie beneath the eyes, becomes infolded and gives rise to the so-called **white bodies** (Fig. 292).

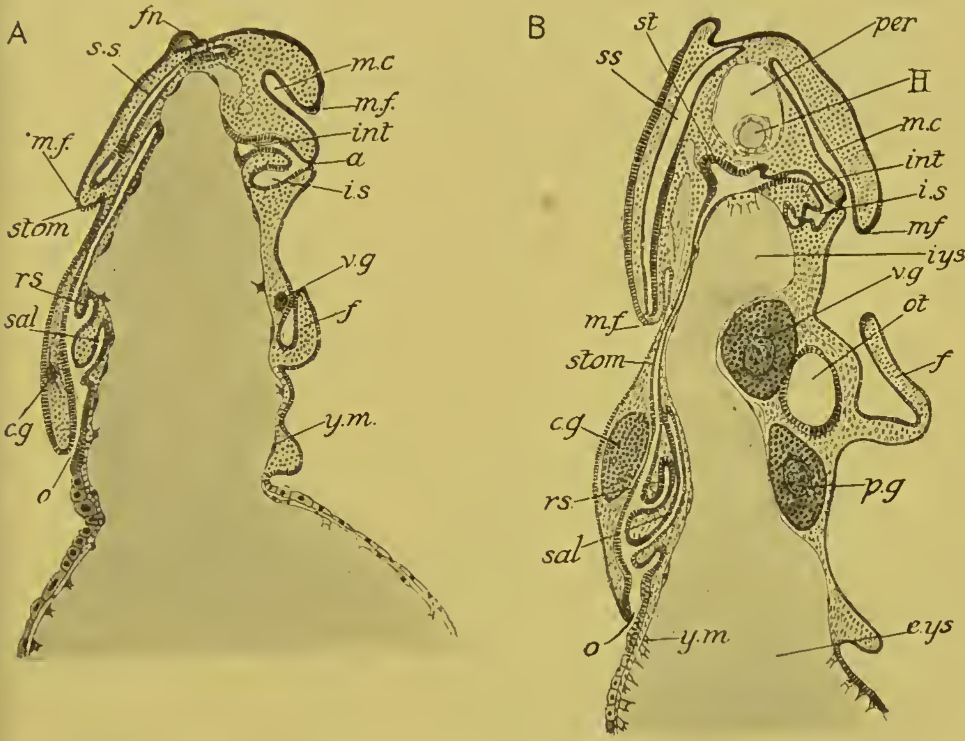

FIG. 293.—Sagittal sections of two embryos of *Loligo vulgaris* to illustrate the formation of internal organs. (After Korschelt.)

A, Section of younger embryo with incomplete alimentary canal. B, Section of older embryo in which stomodaeum and mid-gut have joined. *a*, anus; *c.g*, cerebral ganglion; *e.y.s*, external yolk-sac; *f*, funnel; *fn*, fin rudiment; *H*, heart; *int*, intestine; *i.s*, ink sac; *i.y.s*, internal yolk-sac; *m.c*, mantle-cavity; *m.f*, mantle-fold; *o*, mouth; *ot*, otocyst; *p.g*, pedal ganglion; *per*, pericardium; *r.s*, radula sac; *sal*, salivary gland; *s.s*, shell-sac; *stom*, stomodaeum; *v.g*, visceral ganglion; *y.m*, yolk-membrane.

The internal changes which occur are best made out by combining the views obtained by sagittal sections with those obtained by horizontal sections through the embryo. The most marked features of the stage which we are discussing are the appearance of the **coelomic cavities** and of the **blood spaces**, both of which arise as splits in the mesoderm. The coelomic cavities contain a clear fluid, and have a definite epithelial arrangement of the cells forming their walls; whilst the blood spaces contain an albuminous serum which stains, and their walls are often irregular and in some places formed only by the yolk-membrane.

The coelom arises as two vesicles lying beneath the mid-gut. Each of these vesicles becomes divided by a constriction into a dorsal part, which is the rudiment of the **pericardium,** and a ventral part, the rudiment of the **kidney**; the connection between the two, though narrow, persists, and forms the **reno-pericardial canal.**

Beneath the shell-sac, on the posterior slope of the embryo, a wide blood space arises whose cavity is traversed by cords of mesenchyme. This is the **posterior sinus** (Fig. 294). It extends anteriorly round the sides of the gut and there constitutes the two forks of the **vena cava.** From this a branch extends into the rudiment of the gill, which is the beginning of the **branchial heart.** In front, these forks unite beneath the gut to form the unpaired **vena cava.** The **heart** arises independently of this sinus as two hollow tubes lying internally between the coelomic rudiments. Behind, these tubes unite to form the **ventricle,** but in front, where kidney and coelom join, they diverge so as to form the **auricles** (Fig. 295).

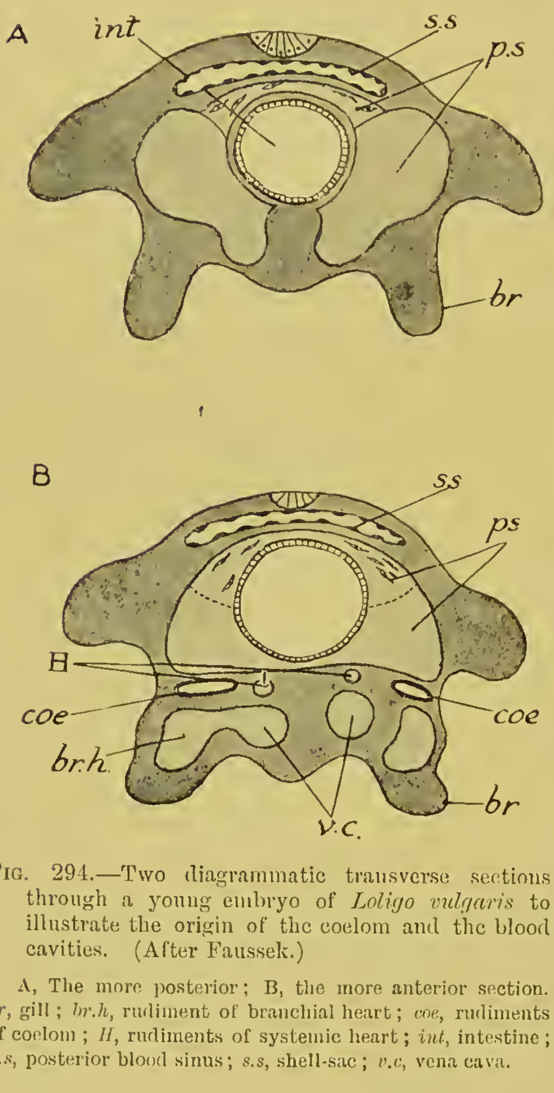

FIG. 294.—Two diagrammatic transverse sections through a young embryo of *Loligo vulgaris* to illustrate the origin of the coelom and the blood cavities. (After Faussek.)

A, The more posterior; B, the more anterior section. *br*, gill; *br.h*, rudiment of branchial heart; *coe*, rudiments of coelom; *H*, rudiments of systemic heart; *int*, intestine; *p.s*, posterior blood sinus; *s.s*, shell-sac; *v.c*, vena cava.

In the next period of development, the end of which is represented in Fig. 296, the embryo becomes as large as the external yolk-sac, and the **funnel** is definitely constituted by the union of the free edges of the folds in the mid-ventral line. The arms have now acquired suckers and have extended round the head to the mid-dorsal line, so that the encircling of the head by the fore foot is completed. The **anterior chamber** of the eye is formed as an ectodermic fold, which arises from the base of the eye-stalk and encloses it. In this way the outer surface of the adult head is formed and the eye-stalks are no longer prominent. This enveloping fold we may term the **corneal fold.** From the spot where the primary eye vesicle closed the rudiment of the outer segment of the lens is formed as an external secretion. The **eyelids** are constituted by a pair of folds similar to those which wall in the anterior chamber, but lying outside the latter. Lying inside the anterior chamber is still another pair of folds which constitute the **iris.**

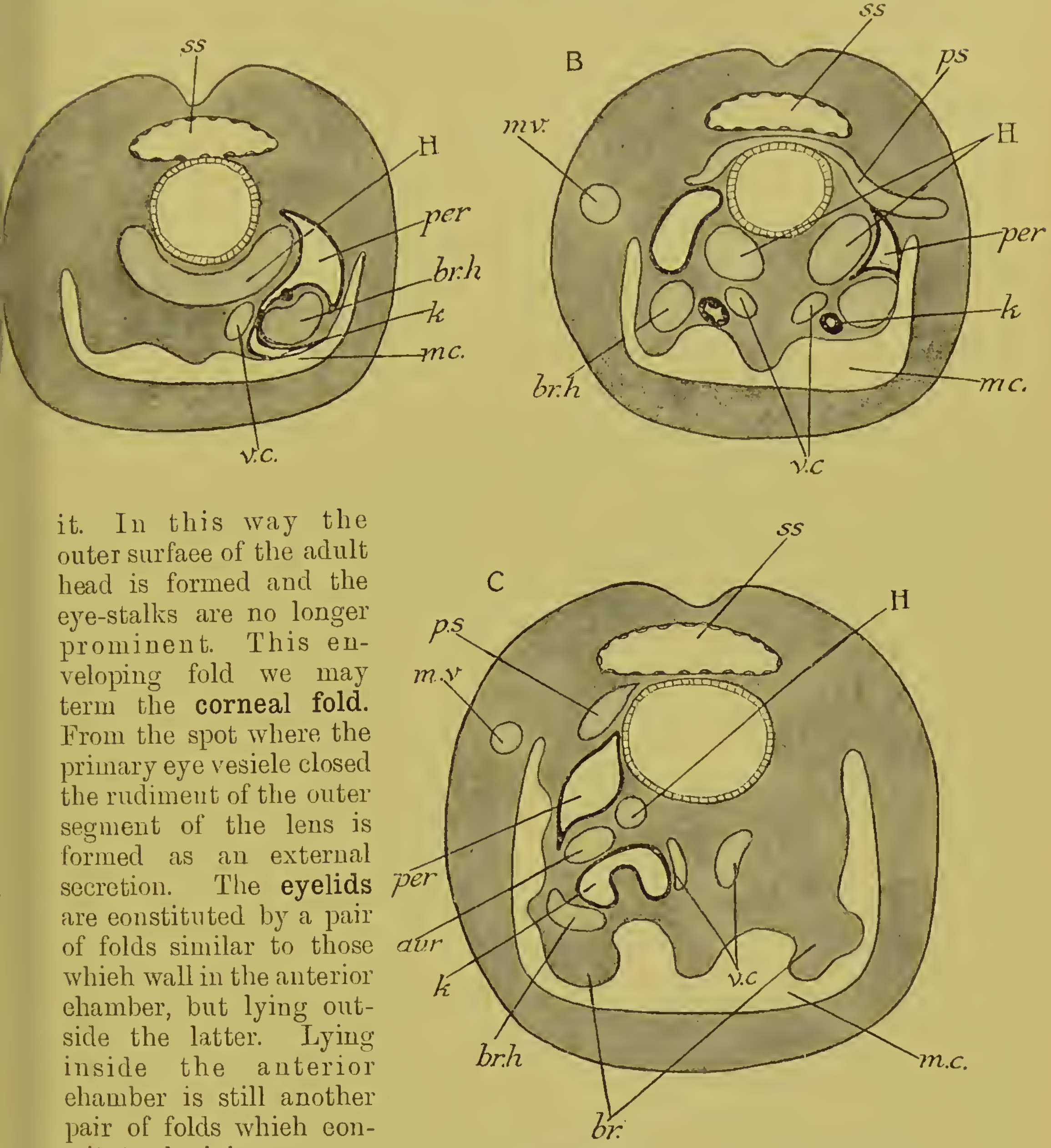

Fig. 295.—Diagrams of three transverse sections through an embryo of *Loligo vulgaris*, much older than that represented in Fig. 294. (After Faussek.)

A, The most posterior; C, the most anterior of the three sections. Letters as in previous figure. In addition, *aur*, auricle; *k*, kidney; *m.c*, mantle-cavity; *m.v*, mantle-vein; *per*, pericardium.

The alimentary canal, which had become a completed tube in the previous period, now shows further development. The **radula sac** becomes sharply marked off from the stomodaeum. In front of it two ventral diverticula grow out, which are the rudiments of the **salivary glands.** The mid-gut becomes differentiated into a **stomach** and an **intestine,** and the diverticulum which forms the ink sac opens into the latter. The **liver** arises as two lateral outgrowths of the stomach. The surfaces of these outgrowths become folded, and this is the first indication of the formation of the **liver tubules.**

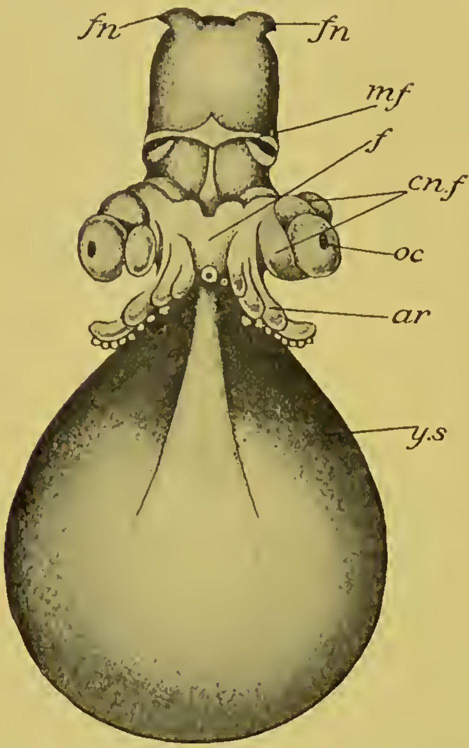

FIG. 296.—Embryo of *Loligo vulgaris* at the period when the funnel is formed, viewed from behind. (After Korschelt.)

ar, arms; *cn.f*, corneal fold which envelops the eye-stalk, and forms the outer chamber of the eye; *f*, completed funnel; *fn*, fins; *m.f*, mantle edge; *oc*, eye; *y.s*, yolk-sac.

The coelom, which is already constricted into kidney and pericardium, now increases greatly in volume. The two pericardial rudiments fuse behind and enclose the two rudiments of the heart; these latter likewise fuse together. In front the pericardial rudiments remain separate and are applied to the paired rudiments of the heart which here constitute the auricles. The paired portions of the pericardium communicate with the kidneys and give rise to the **reno-pericardial canals.** Behind, the single pericardium grows backward and extends into the growing **genital organ,** which becomes divided up into the **genital folds.** This portion of the pericardium becomes, later, divided off from that surrounding the heart and forms the **genital coelom** (Fig. 297).

The kidney sacs develop a high columnar epithelium on their inner walls, where they are in contact with the forks of the vena cava; the epithelium lining their outer walls becomes very thin. The posterior sinus is reduced to very small dimensions by the expansion of the shell-sac, and is cut off from the vena cava. The portions of the vena cava which extend into the gills and constitute the **branchial hearts,** develop great thickenings of their walls on one side. These thickenings, as experiment has proved, are excretory in nature and consist of vacuolated cells; they are covered externally by thin peritoneal epithelium where they touch the coelom. These are of course the **appendages** of the branchial heart.

The **cartilage** so characteristic of Cephalopoda is formed by the modification of mesodermic connective tissue, and is first visible in the neighbourhood of the foot. The **chromatophores** likewise, according to Faussek, are of mesodermal origin. During this period the whole of the ectoderm, except that covering the inner surfaces of the arms, undergoes mucous degeneration—*i.e.* the cytoplasm of its cells degenerates into slime and is cast off. How the adult ectoderm is regenerated Faussek was unable to determine.

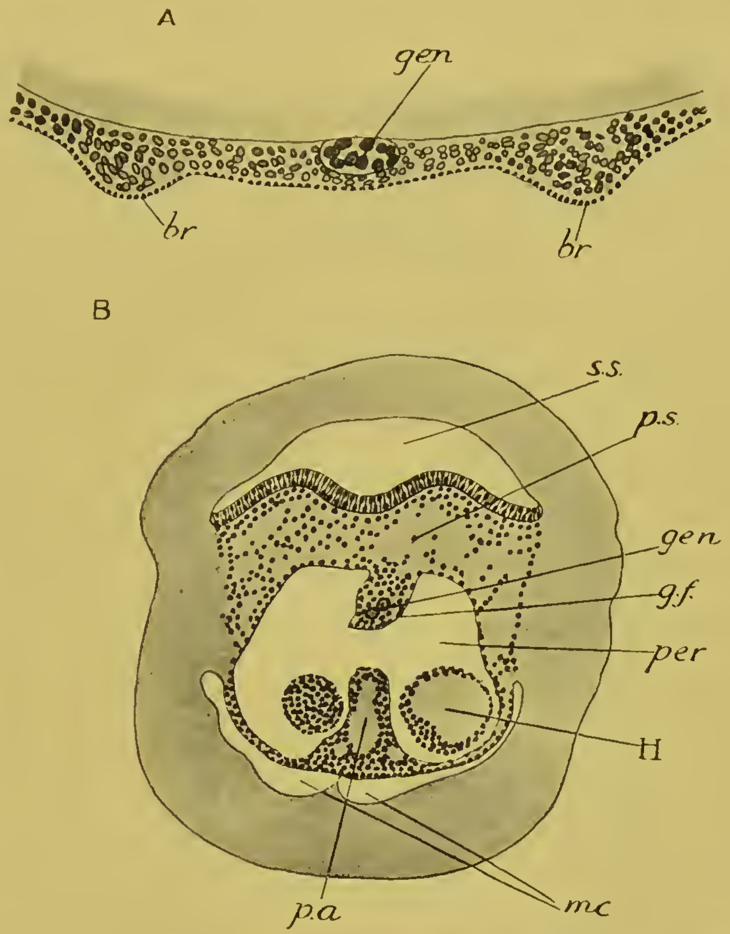

FIG. 297.—Transverse sections of embryos of young cuttle-fish, illustrating two stages in the development of the genital organs. (After Faussek.)

A, Section through young embryo of *Loligo vulgaris*, showing the first appearance of the genital cells in the mesoderm. B, Section through older embryo of *Sepia officinalis*, showing the migration of these cells into a fold projecting into the coelom. *br*, rudiments of gills; *gen*, genital cells; *gf*, genital fold; *H*, heart; *m.c*, mantle-cavity; *p.a*, posterior aorta; *per*, pericardium; *p.s*, posterior sinus; *s.s*, shell-sac.

At the time that the external yolk-sac is absorbed, a large diverticulum, the **spiral caecum**, grows out from the stomach; and this circumstance, together with the enlargement of the internal yolk-sac, into which some of the yolk from the external sac passes, is responsible for the almost entire suppression of the cavities of the kidneys and genital organs which takes place at this period. Later, when the yolk is finally absorbed, the kidneys and pericardium reacquire their cavities, and then the kidneys become fused together in the mid-ventral line; this fusion is characteristic of *Loligo*, and does not occur in *Sepia*.

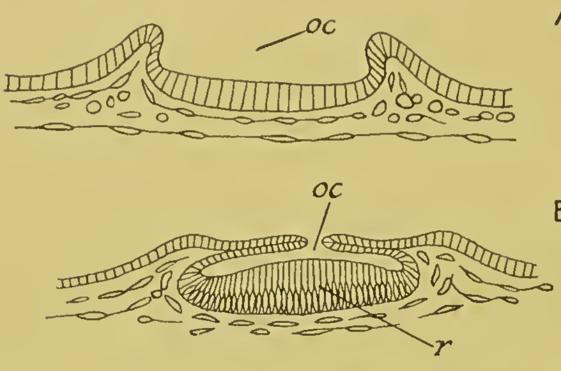

Fig. 298.—Two early stages in the development of the eye of *Loligo vulgaris* seen in transverse section. (After Lankester, from Balfour.)

oc, eye-cup; *r*, rudiment of retina.

By far the most complicated organ in the Cephalopod is the eye, the general features of the development of which have already been described. Some details may now be added. As soon as the primary eye pit closes the **inner segment** of the lens begins to be formed. It first appears as a thin cuticle spreading over a considerable portion of the inner surface of the closed eye-sac, but it becomes thickened at one point in the centre, and

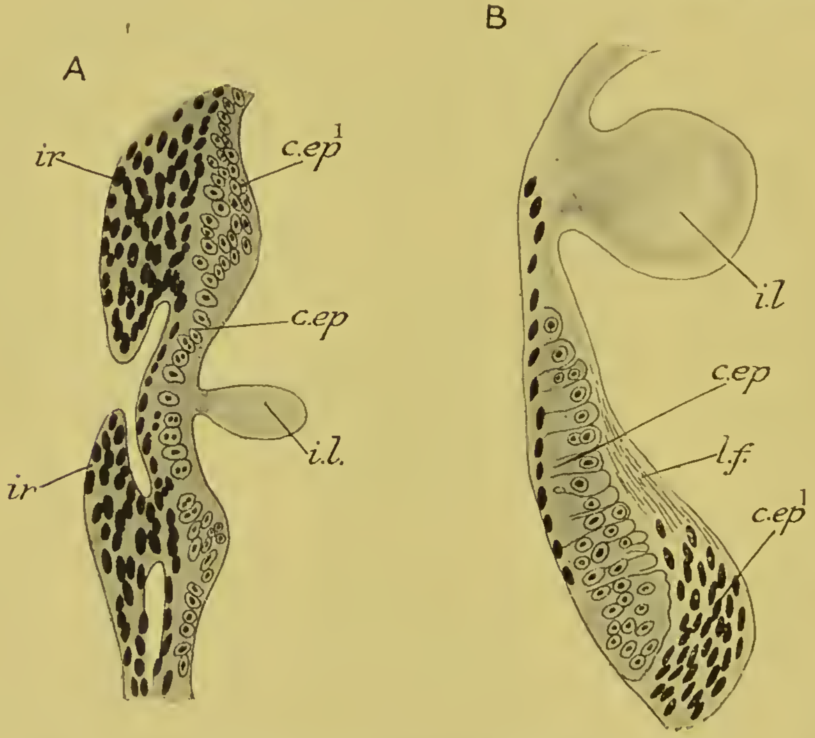

Fig. 299.—Sections through the developing eyes of young cuttle-fish to show the development of the lens. (After Faussek.)

A, Section through the eye of *Loligo vulgaris*. B, Section through the eye of *Sepia officinalis*. *c.ep*, large cells of the corpus epitheliale; *c.ep*^{1}, small cells of the periphery of the corpus epitheliale which grow over the larger cells and secrete the fibres of the lens; *i.l*, inner segment of the lens; *ir*, iris; *l.f*, lens fibres.

projects inwards as a rod-shaped structure. The cells forming this part of the wall of the eye-sac are enlarged and cubical, whilst those forming the more peripheral portions of the eye-sac wall are small. *The large cells, which secreted the primary part of the lens, disappear in the centre, probably as a result of lens secretion;* towards the sides they persist as the characteristic cells of the **corpus epitheliale** of the **ciliary body**. The further growth of the lens is effected by means of the small cells forming the more distant portions of the wall of the eye-sac. These cells grow forward on each side towards the lens, as a kind of fold overspreading the large cells of the corpus epitheliale; and by them the lens is added to, both in thickness and depth.

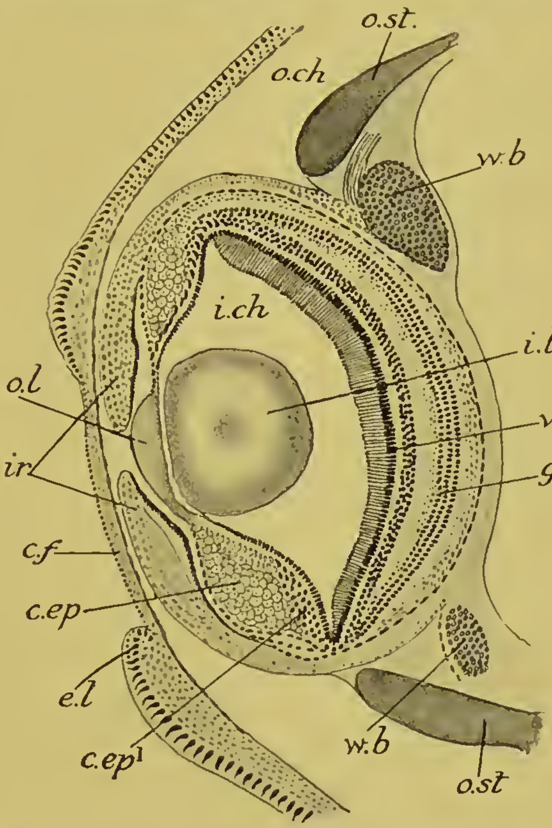

FIG. 300.—Transverse section of the eye of a nearly ripe embryo of *Sepia officinalis*. (After Faussek.)

c.ep, epithelial body; *c.ep*^{1}, small cells of *c.ep* forming lens capsule; *c.f*, corneal fold constituting the outer wall of the outer chamber of the eye; *e.l*, thickening, the rudiment of the lower eye-lid; *g.c*, ganglion cells of the retina; *i.ch*, inner chamber of the eye; *i.l*, inner segment of the lens; *ir*, iris; *o.ch*, outer chamber of the eye; *o.l*, outer segment of the lens; *o.st*, eye-stalk; *v.c*, visual cells of the retina; *w.b*, mesodermal rudiment of the white body.

The **iris folds** and the outer segment of the lens are formed before the fold which walls in the anterior chamber of the eye, and constitutes the **corneal fold**, is formed. The outer segment of the lens is formed like the inner segment; at first it is a cuticle which thickens in the centre, then the cells beneath this thickening disappear and it is added to by the more lateral cells.

If this description be followed it is clear that the primary eye vesicle, with its contained inner segment of the lens, corresponds to the eye as we find it in Gastropoda; and that the outer segment of the lens and the outer chamber of the eye are subsequent additions. The ciliary body consists of the adpressed posterior wall of the outer chamber and anterior wall of the inner chamber of the eye.

The hinder wall of the primary eye vesicle forms the retina. This consists at first of a single layer of columnar ectodermal cells with the nuclei at different levels, bounded externally by a basement membrane. Laterally it is continuous with the layer of small cells which forms the lens. Soon the single layer constituting the retina changes into many layers of small rounded cells; of these the outer layers begin to pass outwards through the basement membrane, and they constitute the nervous layer of the retina. From the innermost layer of cells **visual rods** grow out (*v.c*, Fig. 300), pointing into the cavity of the eye-sac; but these cells do not all undergo this transformation; alternating with the visual cells are cells which secrete pigment. The inner portions of the visual cells, that is, the portions turned towards the cavity of the eye-sac, and these pigment cells, alone retain their primary position with regard to the basement membrane. The nervous portion of the retina is thus seen to consist of two layers of nuclei with a clear space between them (almost certainly occupied by dendrites of the nerve cells), and the whole presents a striking analogy to the layers of cells in the human retina, except that the layers occur in the reverse direction so far as the incidence of light is concerned.

GENERAL CONSIDERATIONS ON THE ANCESTRAL HISTORY OF MOLLUSCA

When we review the account of the development of Mollusca given in this chapter, certain facts stand out clearly. First, in the early larvae of *Patella*, *Dentalium*, and *Dreissensia* we are evidently dealing with a single type, and this type must be classed as a **Trochophore larva**, similar in all essentials to the Trochophore larva of Annelida. Therefore the common ancestral group from which Gastropoda, Scaphopoda, Pelecypoda (and we may add Solenogastres) spring, must have had a Trochophore larva. In a word, all Mollusca are thus shown to be descended from an ancestor represented by the Trochophore, *i.e.* the same ancestor as gave rise to the Annelida.

What, it may be asked, was the factor which caused two families of the descendants of this ancestor to diverge so widely from one another in structure? We must surely look for this factor solely in a divergence of modes of life. Now, the fundamental type of habits common to all Annelida is a burrowing mode of existence; and from that, coupled with a wriggling method of locomotion during their occasional excursions into the upper water, we were able to deduce the main peculiarities of their adult structure. But the habits of

Mollusca do not lend themselves to such easy generalization. The Pelecypoda and Scaphopoda burrow, the Gastropoda crawl, and the Cephalopoda propel themselves by projecting squirts of water through the funnel.

But if we take into account Drew's statement (1899) that *Yoldia* —surely one of the most primitive of Pelecypoda,—when just metamorphosed, glides over the mud by means of its cilia, we might be inclined to conclude that the primitive habits of the original Mollusca consisted in crawling or gliding over the surface, in contradistinction to the burrowing mode of life adopted by primitive Annelida. It is highly probable that the most primitive living Cephalopod, *Nautilus*, in which the constituent folds which make up the funnel are not united, can flatten out this organ and crawl.

But if we are entitled to conclude that the Trochophore larva represents the common ancestor of Annelida and Mollusca, we must regard the various Veliger larvae as representing an anticipation of adult conditions; a telescoping of development, in all respects similar to that shown by the post-trochophoral stages of development in Annelids.

The Veliger of Gastropoda, with its spirally twisted shell, can hardly represent an ancestral stage; because, as we have seen, the unequal growth of the mantle edge which causes the twisting is most plausibly explained by the overbalancing of a tall visceral hump, such as would surely occur in an animal crawling over uneven ground, not in one which was free-swimming. The Veligers of Pelecypoda and Scaphopoda exhibit in the free-swimming stage the distinguishing adult characters of their respective groups. As has been mentioned several times already, this reflection of adult characters into successively early stages of life-history is a phenomenon which meets us everywhere in embryology, and it is one of the most suggestive features in the whole process of development.

Turning now to the development of Cephalopoda, it is at first sight difficult to find any points of contact whatever between their development and that of other Mollusca. Thus, the gills are amongst the earliest organs to be formed in Cephalopoda, whilst they are the latest in Gastropoda and Pelecypoda. All trace of the Trochophore stage has been eliminated from Cephalopod ontogeny, and there is nothing corresponding to the veliger stage. Even the early history of the shell must be greatly hastened through, for the shell-sac and shell gland are two different things. Lankester (1890) has pointed out that, in the later stages of the development of the snail (*Helix*), a large ventral protrusion of the foot filled with yolk is produced. This he rightly compares to the external yolk-sac of the Cephalopod embryo, for this certainly represents a median protrusion of the foot, since the rest of the foot is formed all round it. We have here, however, a case of analogous development, not of real homology, for the heavily yolked egg of the asymmetrical snail has not been derived from the heavily yolked egg of the Cephalopod, or *vice versa*.

If, and when, the development of *Nautilus* is worked out, we shall probably gain points for comparison of the development of Cephalopoda with that of other Mollusca; in the meantime we can only conclude that the accession of large stores of nourishment has almost obliterated the traces of ancestral history in their development, leaving only the most general resemblance in the formation of the layers and the development of the sense-organs, as links between them and other Mollusca. In fact, the reflection of the development of organs which become important in adult life, into successively earlier periods of development, termed by Lankester **heterochrony**, has, in Cephalopoda, reached its maximum.

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CHAPTER X

PODAXONIA

Classification adopted—

Podaxonia (Gephyrea nuda) { **Sipunculoidea**
Phoronidea

In the old group of the Gephyrea, which used to be regarded as a subdivision of the Annelida, there were included several families or sub-orders of such diverse structure that it has been recently customary to separate them entirely from each other and to regard them as belonging to quite distinct phyla. Of these families one, the Echiuroidea, is undoubtedly to be regarded as a modified group of Polychaeta; and about another, the Priapuloidea, nothing can be said until the development has been worked out, and we know more about the adult anatomy of its members. A third group, the Sipunculoidea, constituting the Gephyrea nuda, agrees with Annelida and Mollusca in possessing a Trochophore larva, and hence must be regarded as descendants of the same Ctenophore-like ancestor, from which, as we have seen reason to believe, these two phyla originated. They differ, however, from both Annelida and Mollusca in the fact that the principal extension of the body takes place in a direction almost at right angles to the line joining mouth and anus, and furthermore in a ventral direction. Hence the name **Podaxonia**, coined for them by Ray Lankester (1890) with his customary insight.

It is probable that the group of Ectoproct Polyzoa is allied to the Podaxonia, but the full proof of that is a matter to be settled by future investigation. The group of the Phoronidea, however, constituting the old division of Gephyrea tubicola, is almost certainly closely allied to the Podaxonia, of which it will be considered a subdivision. Evidence in favour of this view will be offered in this chapter.

PHASCOLOSOMA

The genus *Phascolosoma* has representative species on both sides of the Atlantic. The cell-lineage and larval development of the

American species *P. gouldii* has been worked out by Gerould (1907), who also confirmed his results by work done on the European species *P. vulgare.*

The eggs of the American species, when they mature, are dehisced into the coelom and pass into the nephridia. They are finally laid in the sea and there fertilized. They are provided with a strong "chorion" or "yolk-membrane," which persists until the close of embryonic and the beginning of larval development. The spermatozoon penetrates this membrane through a micropyle. After the eggs have been fixed in picro-sulphuric acid, it is possible to dissolve the chorion by exposing them to Laburraque's solution for two hours, and according to Gerould no harm is done to the egg itself by this treatment.

The cleavage reminds us in many ways of that of *Dentalium.* The egg divides into the usual four macromeres, but D is, from the first, very much bigger than its sisters A, B, and C. It has, in fact, five times the volume of any one of its three sisters. In the 8-cell stage a first quartette of micromeres is formed, and these are relatively large cells, as big as the smaller macromeres.

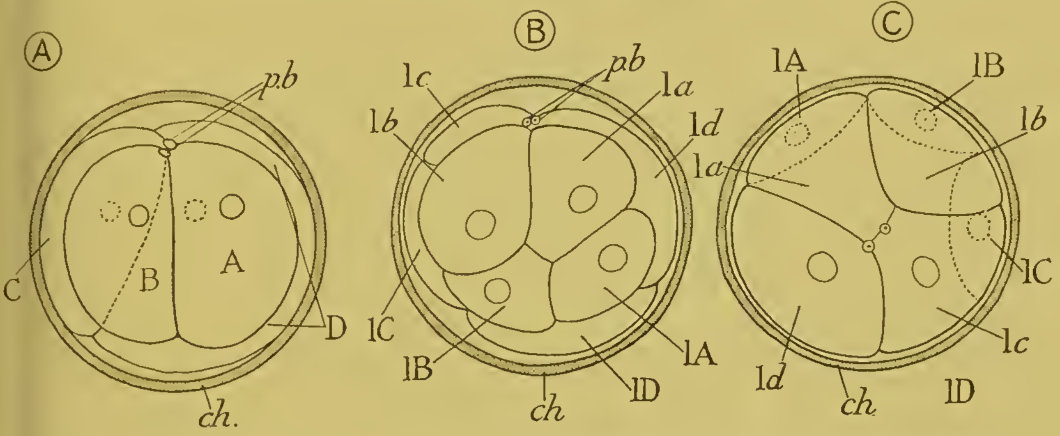

FIG. 301.—Early segmentation stages of the egg of *Phascolosoma gouldii.* (After Gerould.)

A, 4-cell stage viewed from the side. B, 8-cell stage viewed from the side. C, 8-cell stage viewed from above. *ch*, chorion; *p.b*, polar bodies.

In the 16-cell stage 1a, 1b, 1c, and 1d divide as usual into $1a^1$, $1b^1$, $1c^1$, and $1d^1$, and $1a^2$, $1b^2$, $1c^2$, and $1d^2$ respectively. These two sets of cells are about equal in size, and they are larger than the residual macromeres 2A, 2B, and 2C. Of the second quartette of micromeres which, with these macromeres, form the lower half of the egg, 2a, 2b, and 2c are small, but 2d and its sister cell, the residual macromere 2D, are both enormous and of about equal size. In attaining the 32-cell stage, the upper eight cells of the egg divide equally, so that the quartettes of cells $1q^{11}$, $1q^{12}$, $1q^{21}$, and $1q^{22}$ are all of about equal size. So far as the second quartette of micromeres are concerned, each divides into a small upper and lower larger cell. The residual macromeres 2A, etc., give off the third quartette of micromeres; 3a, 3b, and 3c being formed first and 3d later. They are all comparatively small cells (Fig. 302).

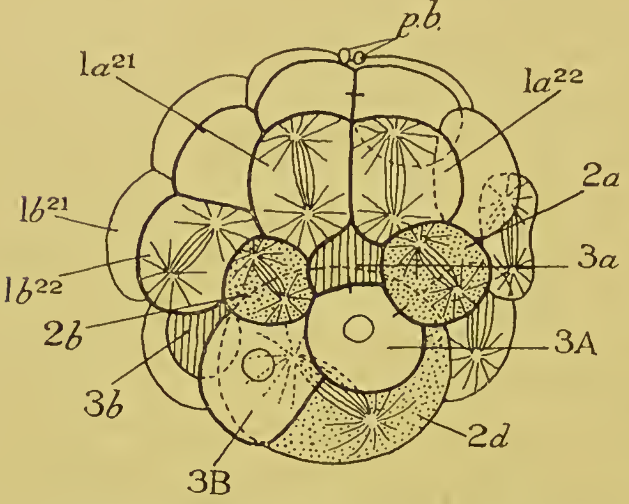

FIG. 302.—Later stage in the segmentation of the egg of *Phascolosoma gouldii*, viewed from the posterior aspect. (After Gerould.)

Cells belonging to the second quartette are dotted; those belonging to the third quartette are ruled with vertical lines.

The upper half of the egg continues to divide more rapidly than the lower half. $1q^{11}$ divides into $1q^{111}$, the **apical cells**, and $1q^{112}$, which are the so-called "**peripheral rosettes**" or the **Annelidan cross**, whilst the so-called "**intermediate girdle cells**," $1q^{12}$, divide into $1q^{121}$, the basal, and $1q^{122}$, the intermediate cells of the arms of the "**Molluscan cross.**" $1q^{21}$ and $1q^{22}$ also each divide, so that in each quadrant of the egg there are four daughters of $1q^2$, and these cells are, of course, as in Mollusca and Annelida, the **primary trochoblast** cells.

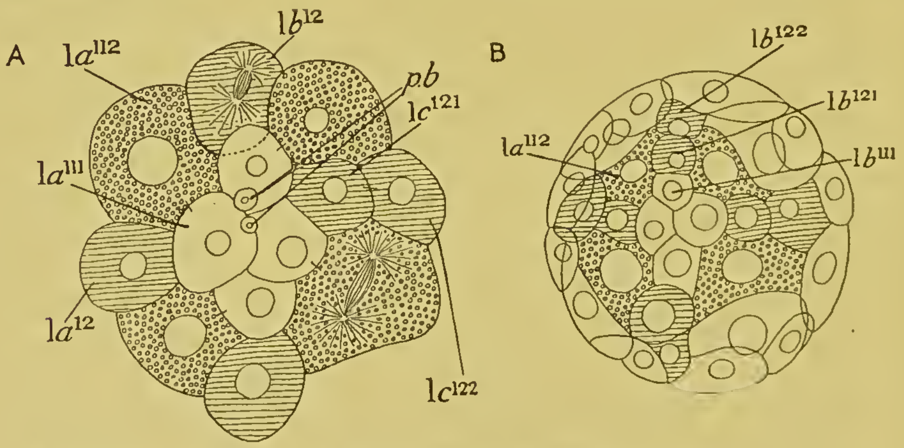

FIG. 303.—Two views of the apical region of the segmenting egg of *Phascolosoma vulgare*. (After Gerould.)

The apical and the prototrochal cells are left white. The "peripheral rosettes" or "Annelidan cross" cells are covered with circles, whilst the "intermediate girdle cells" or "Molluscan cross" are ruled with horizontal lines. A, early stage. B, 48-cell stage. *p b*, polar bodies.

These cells in *Phascolosoma* are very large and extend backwards so as to overlap and cover the cells of the second and third quartettes. They become thickly covered with somewhat small cilia. The three intermediate cells of the Molluscan cross $1a^{122}$, $1b^{122}$, $1c^{122}$, also acquire cilia and are incorporated in the prototrochal girdle. They are the **secondary trochoblasts** (Fig. 303). Of the fourth quartette of micromeres 4d is formed long before the others, and, as usual, immediately divides into right and left sisters $4d^r$ and $4d^l$, which are the **mother cells of the mesoderm.** The residual macromeres eventually form a plate of **endoderm** which is situated beneath the "b" arm of the Molluscan cross.

As in *Patella* and *Dentalium* the process of gastrulation begins by the sinking-in of this plate, and this in-sinking is caused by a

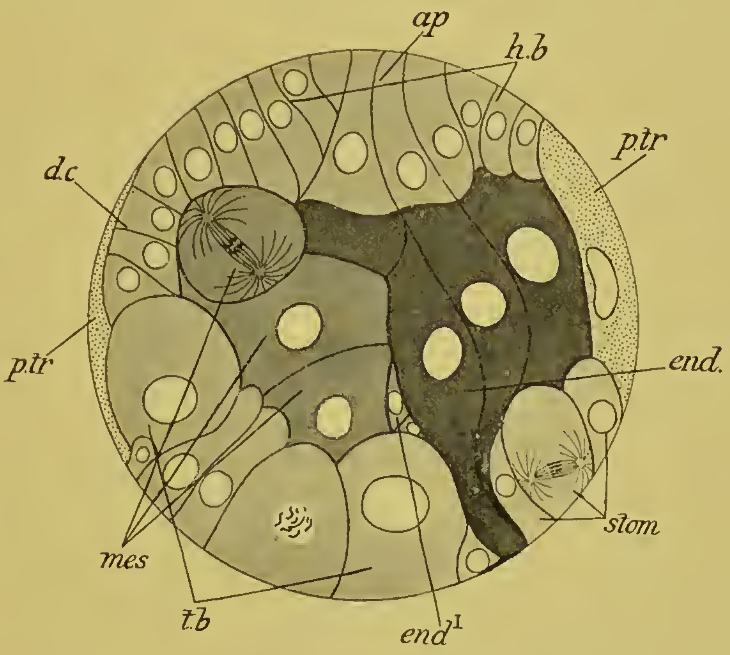

FIG. 304.—Nearly sagittal section of an embryo of *Phascolosoma vulgare* at the stage when gastrulation is beginning. (After Gerould.)

a.p, apical plate; *d.c*, dorsal cord; *end*, endodermal cells derived from residual macromeres; *end*.^{1}, accessory endodermal cells, derived from the mesodermal band; *h.b*, head-blastema; *mes*, mesodermal band; *p.tr*, prototrochal cells; *stom*, cells which give rise to the stomodaeum; *t.b*, cells forming the trunk-blastema.

change of shape in its component cells. These elongate and become flask-shaped, the bulb of each flask passes into the segmentation-cavity or **blastocoele** whilst the neck remains for a time connected with the surface (Fig. 304).

Further divisions at the upper pole of the egg result in the production of a diamond-shaped apical plate of cells surrounding four central cells, the apical cells, which acquire long stiff hairs and form the apical sense-organ. The cells surrounding this apical plate then begin to sink inwards so as to form a ring-shaped invagination which is, as a matter of fact, the **head-blastema.** The **trunk-blastema** lies on the ventral surface, behind where the blastopore is situated and where the stomodaeum is eventually formed. It is a triangular plate of cells with the apex directed forwards and is formed of descendants of 2d. Trunk-blastema and head-blastema are connected in the mid-dorsal line by a narrow cord of cells—the **dorsal cord** (*d.c.* Fig. 304).

The mother cells of the mesoderm, by this time, have each given rise to an anterior band of four cells and to a minute posterior cell, which lies against the endoderm, and which, as in Mollusca, probably gives rise to the intestine. The closure of the blastopore is effected partly by the forward growth of the trunk-blastema and partly by the in-sinking of the descendants of 2a, 2b, and 2c which give rise to the **stomodaeum.**

The embryo becomes a larva by beginning to swim. This happens about twenty-four hours after fertilization of the egg. The larva does not burst the egg-membrane, but carries it about with it.

The **prototroch,** composed of the primary and secondary trochoblasts, is a broad belt of cells covered with minute cilia. There is a well-marked **metatroch** consisting of a girdle of long cilia. Between prototroch and metatroch is situated the opening of the stomodaeum, which is surrounded by a special girdle of small cilia. In front of the apical plate, to the right and left of the middle line, **eye-spots** are found (*oc,* Fig. 305); these are situated just above the region where the cells are being invaginated to form the head-blastema.

Until thirty-six hours after fertilization have elapsed, the larva remains spherical. After that time the posterior portion of the body elongates more rapidly than the anterior portion, and at the beginning of the third day, in the case, at any rate, of *Phascolosoma vulgare,* it sinks to the bottom. At this period the egg-membrane is at last shed, and underneath it a fine cuticle is now to be seen, which has been mistaken for the persisting egg-membrane but is in reality quite distinct from it.

Before, however, this happens, very considerable changes occur in the Trochophore larva. The appearance of the apical plate changes, since the sense-organ appears to move to its anterior edge; this is due to the anterior part of the plate becoming invaginated to form the **cerebral ganglion.** Round the edge of the apical plate is found the **prae-oral band of cilia;** this has nothing to do with the prototroch, the cells composing which carry quite minute cilia. Behind the prototroch there is a narrow band of ectoderm from which the **mesectoderm** is formed; that is to say, from this band cells are budded inwards into the blastocoele. Some of these cells are transformed into longitudinal **accessory retractors** (*ret.acc,* Fig. 306), others become changed into circular muscles. The **principal retractors** are formed from the cells of the apical plate which bear the prae-oral circle of cilia. These retractors retain throughout life their insertion into the ectoderm at the point where they originated from the apical plate (*ret.d, ret.v,* Fig. 306).

Before the Trochophore larva sinks to the bottom the rudiment of the **ventral nerve cord** makes its appearance as an unpaired ectodermic thickening. This thickening becomes detached from the ectoderm and sinks inwards, and in *Phascolosoma gouldii* (but not in *Phascolosoma vulgare*) it becomes divided into two to four segments. These may be regarded as **ganglia** of the nerve cord. In *Phascolosoma gouldii* the mesoderm also becomes divided into segments (*mes*^{1}, *mes*^{2}, *mes*^{3}, Fig. 306). The **anus** is formed, about the forty-fifth hour, by a narrow cone of endoderm cells growing out dorsally and becoming attached to a cluster of ectoderm cells which become slightly invaginated. Somewhat later the **coelomic cavity** appears; in *Phascolosoma gouldii* spaces appear in each of the mesodermal segments, which fuse together and form one undivided cavity. In *P. vulgare*, however, in which the mesoderm is unsegmented, the coelom appears from the beginning as an undivided space.

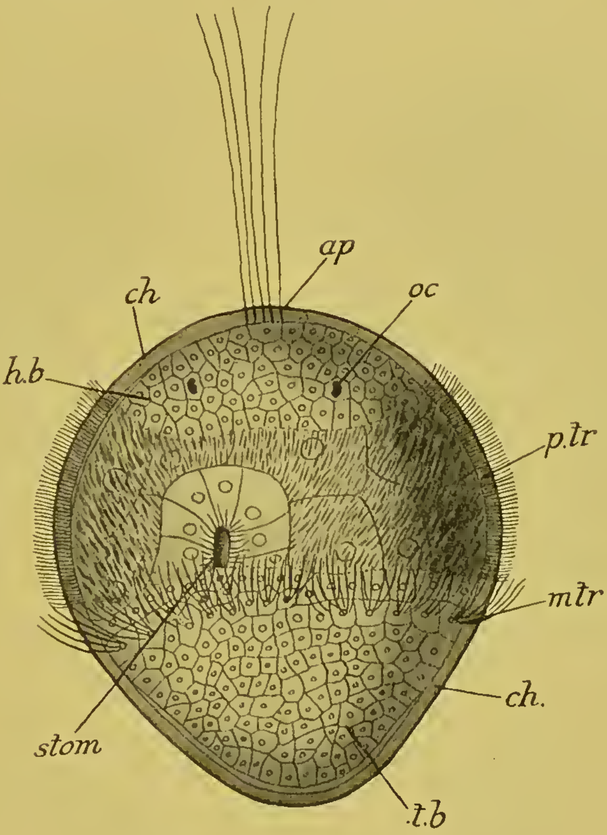

FIG. 305.—A Trochophore larva of *Phascolosoma vulgare* a little more than thirty-six hours old. (After two figures by Gerould combined.)

ap, apical plate with its tuft of cilia; *ch*, chorion still investing the embryo; *h.b*, head-blastema; *mtr*, metatroch; *p.tr*, prototroch; *oc*, eye-spots; *stom*, stomodaeum; *t.b*, trunk-blastema.

When the Trochophore sinks to the bottom the prototroch is got rid of by a most peculiar process. The inner ends of the large cells of which it is constituted break down into yolky granules; these (*y.g*, Fig. 306) are shed into the coelom and are there taken up by amoebocytes and absorbed. In this way, gradually, the whole of the prototroch is disposed of. During this process the larva takes on a cylindrical form with a diminishing ring-shaped swelling in front; this swelling is the disappearing prototroch.

A nerve strand runs from the cerebral ganglion to the apical sense-organ, and from this ganglion originate a pair of muscle cells which run backwards and are inserted into the dorso-lateral region of the skin behind the prototroch. At the sides of the ventral nerve plate there are situated two series of clusters of muscle cells. The more ventral of these run backwards towards the posterior insertion of the retractors; the more dorsal extend forwards to the region between prototroch and post-oral circlet.

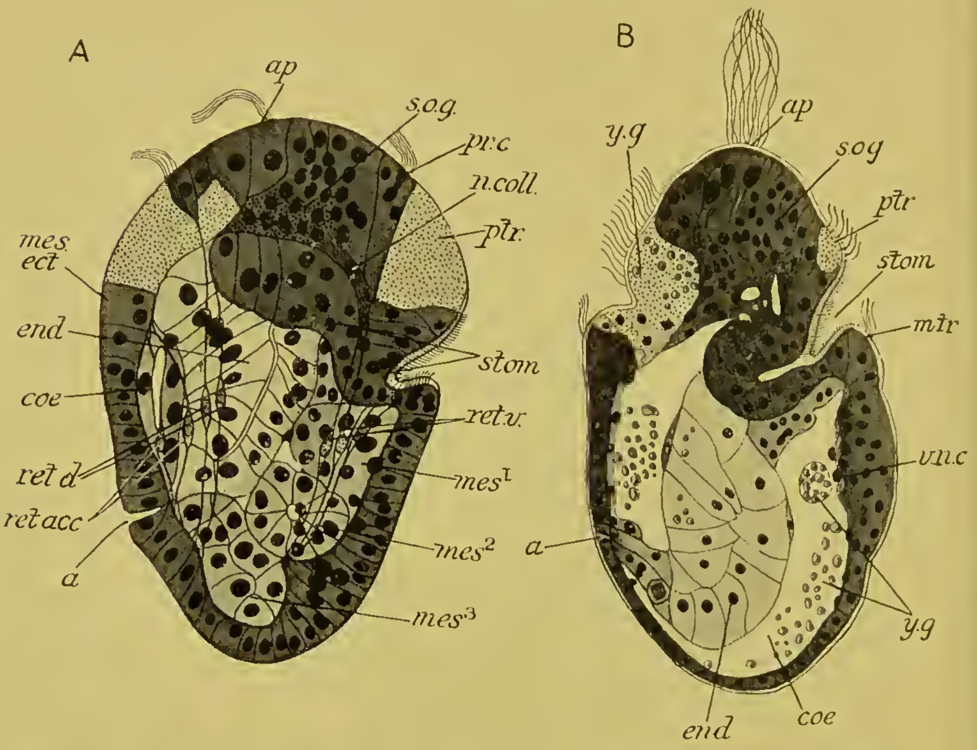

FIG. 306.—Nearly sagittal sections through metamorphosing trochophores of *Phascolosoma*. A, sagittal section to one side of the middle line of the larva of *Phascolosoma gouldii*, about fifty-seven hours old, to show the segments of the mesodermal band. (After two figures by Gerould combined.) B, sagittal section nearly median of the larva of *Phascolosoma vulgare*, about forty-eight hours old. (After Gerould.)

a, anus; *ap*, apical plate; *coe*, coelomic cavity; *end*, endodermic tube; *mes*¹, *mes*², *mes*³, segments of the mesodermic band; *mes.ect*, point of origin of the mesectoderm; *mtr*, metatroch; *n.coll*, nerve collar; *pr.c*, prae-oral circle of cilia; *ptr*, degenerate prototrochal cells; *ret.acc*, accessory retractors; *ret.d*, dorsal retractor muscles formed from ectoderm; *ret.v*, ventral retractor muscles formed from ectoderm; *s.o.g*, supra-oesophageal ganglion; *stom*, stomodaeum; *v.n.c*, ventral nerve cord; *y.g*, yolk-granules, remains of prototrochal cells.

The invagination of the anterior region of the body, so as to form an **introvert**, is begun as soon as the coelomic cavity appears in the mesodermic bands. Then the retractor muscles, whose formation was described above, begin to act and to pull in the whole apical region.

Up till the end of the sixth day there is a freely projecting flattened **prostomium**, ciliated on its under surface. In the second week this prostomium grows out into a dorso-lateral extension on each side, on which **ciliated tentacles** are developed. Beneath the mouth a ciliated under-lip is formed.

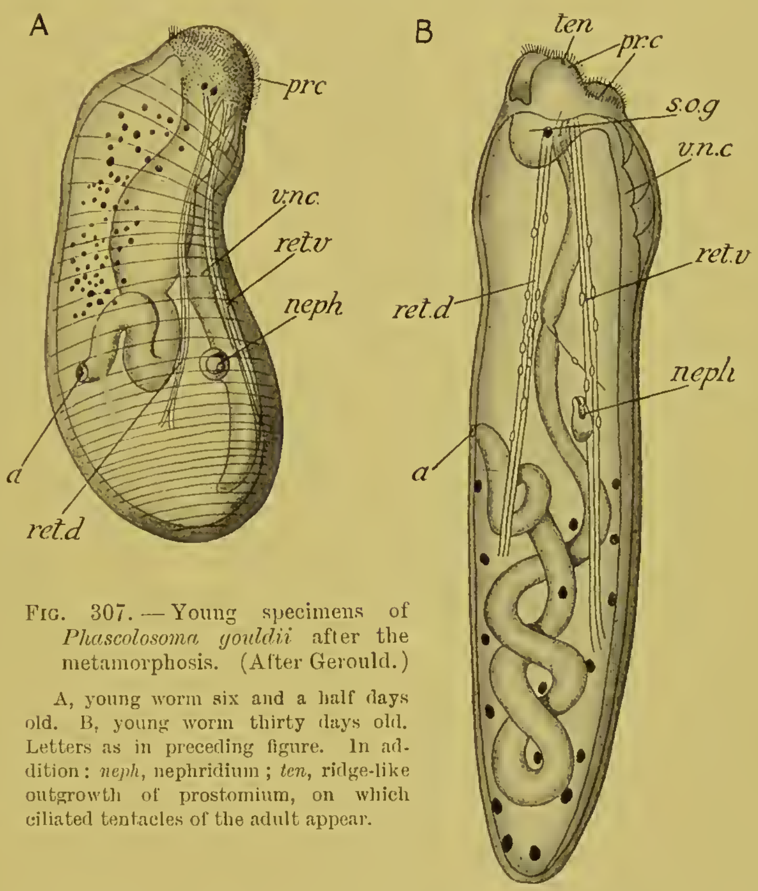

FIG. 307. — Young specimens of *Phascolosoma gouldii* after the metamorphosis. (After Gerould.)

A, young worm six and a half days old. B, young worm thirty days old. Letters as in preceding figure. In addition: *neph*, nephridium; *ten*, ridge-like outgrowth of prostomium, on which ciliated tentacles of the adult appear.

Both species of *Phascolosoma* then develop a circle of hooks round the base of the introvert; these can still be made out as minute hooks in the adult *P. vulgare*, but in *P. gouldii* they entirely disappear. The papillae, so characteristic of the adult skin, appear as oval clusters of ectoderm cells which project slightly inwards towards the coelom.

Large yellow cells (chloragogen cells) are disposed in lines along the coelom. The two **nephridia** (*neph*, Fig. 307) appear to originate as solid ectodermal ingrowths, in each of which a cavity appears later. They come to open into the coelom by the intervention of cells of coelomic origin, from which the internal funnel or **nephrostome** is formed.

As the body grows longer and longer the anus appears to move forwards, but this appearance is simply due to the fact that the part of the body intervening between the apical plate and the anus does not grow nearly so fast as the portion situated behind the mouth, on the ventral surface; *this disparity of growth is the essential characteristic of all Podaxonia.*

If we review the development which has just been described, we shall find ourselves driven to the conclusion that, not only are *Phascolosoma* and its allies descended from the common Ctenophore-like ancestor of Annelida and Mollusca, but that they have diverged from the Annelid stem after the beginnings of segmentation had been acquired, and that they represent one mode in which the descendants of the primitive Annelida were adapted to a burrowing life.

SIPUNCULUS

The development of the well-known Mediterranean genus *Sipunculus* has also been worked out by Hatschek (1883) though not at all in the same detail as Gerould has worked out *Phascolosoma*. It agrees in all essentials of its embryonic and larval history with *Phascolosoma*, the chief differences being the form which the prototroch assumes and the mode of disposing of it.

In *Sipunculus* the prototroch, instead of being represented by sixteen primary prototrochal cells, is represented by a broad mantle of comparatively small cells carrying short cilia. This mantle is, however, incomplete in the mid-dorsal line, where a narrow line of sunken cells connects the head- and trunk-blastema. This line of cells corresponds to the dorsal cord of *Phascolosoma*. When the larva metamorphoses the whole of the mantle is cast off, as in *Nucula* amongst Mollusca, and is not absorbed into the coelom as in *Phascolosoma*.

PHORONIDEA

A form of great interest, which was placed in the old group of Gephyrea under the division Gephyrea tubicola, is *Phoronis*. *Phoronis* is now made the type of a special family, the Phoronidea, which Lankester considers to belong to the Polyzoa, but which we regard as more nearly related to the Sipunculoidea whose development we have just discussed.

Phoronis agrees with the typical Podaxonia in the ventral development of the body, but instead of living in sand and mud it inhabits a leathery tube which it secretes for itself. It possesses, like most Podaxonia, a curved lateral extension of the lips of the mouth bearing ciliated tentacles, but these, instead of being prae-oral as in *Phascolosoma*, are post-oral.

The full embryonic history of Phoronis has not been satisfactorily made out, although a preliminary account of the subject has been given by Caldwell (1883 and 1885), and further work on the subject has been done by Masterman (1898), Ikeda (1901), de Selys Longchamps (1902), and Shearer (1906).

The free-swimming larva of *Phoronis* is termed Actinotrocha, and was regarded as an independent organism before its life-history was known. Its remarkable metamorphosis into the adult form was described by Metschnikoff (1871), while a minute description of the structure of the adult larva was given by Goodrich (1905).

Masterman's paper awakened widespread interest and created a lively controversy. He endeavoured to show that Actinotrocha, like the larva of *Balanoglossus* (p. 575), possessed five coelomic sacs, viz. a prae-oral and two pairs of lateral sacs; and that these sacs were developed as outgrowths from the gut, and that consequently *Phoronis* was allied to the Protochordata, and in particular to *Cephalodiscus*, which has ciliated tentacles like those of the Actinotrocha larva. He even endeavoured to find the homologue of the notochord in two glandular pouches which project forwards from the stomach of Actinotrocha (*gl*, Fig. 311).

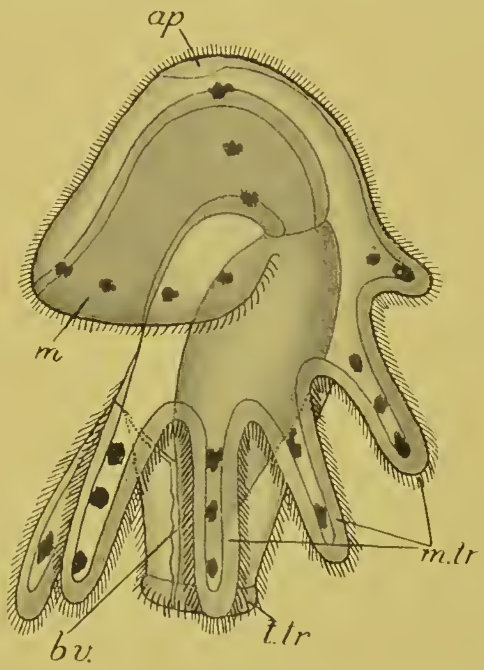

FIG. 308.—Lateral view of the Actinotrocha larva of *Phoronis*. (After Metschnikoff.)

ap, apical plate; *b.v*, blood-vessel; *m*, mouth; *m.tr*, metatroch; *t.tr*, telotroch.

The Actinotrocha larva possesses a hood-shaped prae-oral lobe covered with minute cilia and carrying a thickened apical plate. Somewhat below the centre of its upper surface the prae-oral lobe contains a cavity, called by Masterman the **prae-oral coelom.** Behind the mouth there is an oblique ciliated band, in other words a **metatroch,** which is drawn out into a series of hollow tentacles (Fig. 309). The tentacles contain cavities which open into right and left loop-shaped vessels, situated at the sides of the oesophagus, which were compared by Masterman to the "**collar coelomic cavities**" of *Cephalodiscus*. There are a pair of nephridial tubes which, according to Masterman, open internally into the collar-cavities and are compared by him to the **collar pores** of *Cephalodiscus*. Behind these a pair of coelomic sacs flank the alimentary canal, which correspond to the **trunk coelomic cavities** of *Cephalodiscus*. A ciliated girdle or **telotroch** encircles the hinder end of the larva.

Unfortunately Masterman's fascinating hypothesis has not been sustained by subsequent workers. Thus, Goodrich shows clearly that the so-called prae-oral coelom is merely a portion of the blastocoele, or primary body-cavity, corresponding to the cavity surrounding the gut in a Trochophore larva; and that the nephridia cannot be homologous with collar pores, because they end blindly internally and are beset with solenocytes projecting into the blastocoele; they are in fact archinephridia like those of Annelida. Goodrich admits the existence of collar and trunk coelomic cavities, but Ikeda, de Selys Longchamps, and Shearer deny that these arise as endodermic diverticula.

In justice to Masterman it ought to be noted that Caldwell described the coelom as arising from two posterior outgrowths from the gut (Fig. 310); and though Shearer asserts that the bilobed ingrowth seen by Caldwell is an ectodermic pocket which gives rise to the tubes of the nephridia, yet the fact that the first trace of the coelom seen by him was an unpaired bilobed sac, lying close to the dorsal surface of the hinder end of the gut, renders it possible that after all Caldwell and Masterman are right and that the trunk coelom does arise as a pair of posterior diverticula of the gut, at least in some species. Further work is needed to obtain complete certainty on this point.

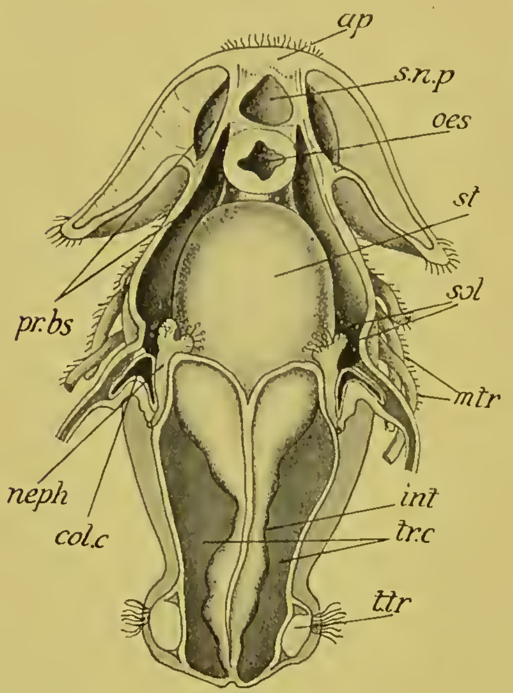

Fig. 309.—Diagrammatic frontal section of the Actinotrocha larva of *Phoronis* (sp^1) captured near Ceylon. (After Goodrich.)

ap, apical plate; *col.c*, collar coelom; *int*, intestine; *mtr*, tentacles of the metatroch; *neph*, nephridium; *oes*, oesophagus; *pr.bs*, prae-oral blood space; *s.n.p*, subneural pit; *sol*, solenocytes of the nephridia; *st*, stomach; *tr.c*, trunk coelom; *t.tr*, telotroch.

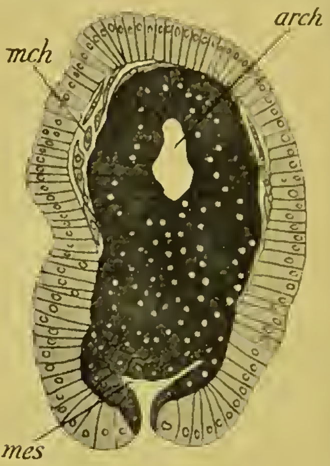

Fig. 310.—Longitudinal horizontal section of the embryo of an Australian species of Phoronis. (After Caldwell.)

arch, archenteron; *mch*, mesenchyme cells; *mes*, diverticula of the archenteron giving rise to the mesoderm of the trunk-cavities according to Caldwell. According to Shearer, an ectodermic pit giving rise to the tubes of the two nephridia.

In any case, however, we fear that Masterman's hypothesis cannot be upheld. Even if the "trunk" coelom does arise in the way which he describes, this does not necessarily prove a close affinity between *Phoronis* and the Protochordata, because we have already seen that the origin of the mesoderm from 4d in Annelidan eggs must be regarded as a modification of such a mode of development, and Erlanger has actually described the mesoderm as arising as a pouch in *Paludina*.

The fatal flaw in Masterman's theory is the absence of a prae-oral coelom in the Actinotrocha larva, and though it is conceivable that the notochord of *Cephalodiscus* should be represented by a paired structure in Actinotrocha, yet the glandular pouches of Actinotrocha have no resemblance to a notochord. The notochord in all Protochordata, and in Vertebrata, is a modification of the *endoderm* into a supporting tissue, by an increase in thickness of the cell-walls of its component cells and the degeneracy of their contained protoplasm. Such a change can be seen in the endoderm of the solid tentacles of the hydroids of *Tubularia*, for instance, as compared with the endoderm of the hollow tentacles of *Hydra*. But the mere fact that the cells composing these glandular pockets in Actinotrocha contain large vacuoles, does not create any special resemblance to a notochord.

On the whole, the early development, so far as it is known, creates the impression of being a modified form of the development described for *Phascolosoma*. In both forms the nephridia arise as ectodermal pockets which, subsequently, after the metamorphosis has been accomplished, acquire openings into the coelom. The ciliated prae-oral lobe of *Phoronis* may be compared to the prototroch of *Phascolosoma*. In both forms the metatroch is prominent.

After the Actinotrocha has led a free-swimming existence for some time, and has increased in size, an invagination of the ectoderm appears on the ventral surface, mid-way between mouth and anus. This pouch increases in depth until it reaches the intestine of the larva, to which it becomes adherent. The intestine increases greatly in length and is thrown into several loops (Fig. 311); the pouch is also thrown into folds as it grows longer.

At length a critical point of growth is reached, at which metamorphosis suddenly supervenes. The ectodermic sac is everted and forms a huge evagination which constitutes the main part of the body of the "worm." As the intestine was attached to the apex of this sac, when this is evaginated the intestine is drawn out in a U shape. The ciliated tentacles of the post-oral band fall off, but from their bases grow out stumps from which the adult tentacles are later developed. The prae-oral lobe disappears, according to Caldwell (1883) it is bodily amputated and falls into the gaping mouth and is there digested. The whole metamorphosis occupies only a quarter of an hour. We ourselves can testify that on one occasion we left an advanced Actinotrocha in a watch-glass, left the room for a short time, and on coming back found a young *Phoronis*.

If Caldwell has given the details of the metamorphosis correctly it is exceedingly difficult to interpret, for his account would seem to imply that the apical plate and subjacent ganglion are sacrificed, in which case the cerebral ganglion of the adult must be a new formation. Now in the metamorphosis of every Trochophore so far studied, the apical plate and the associated ganglion form the head-blastema, and persist through larval life into the adult condition. It is possible that Caldwell has made a mistake in this matter, and that it is the hood in front of the ganglion, which we have already compared to a broad prototroch bearing minute cilia, which is

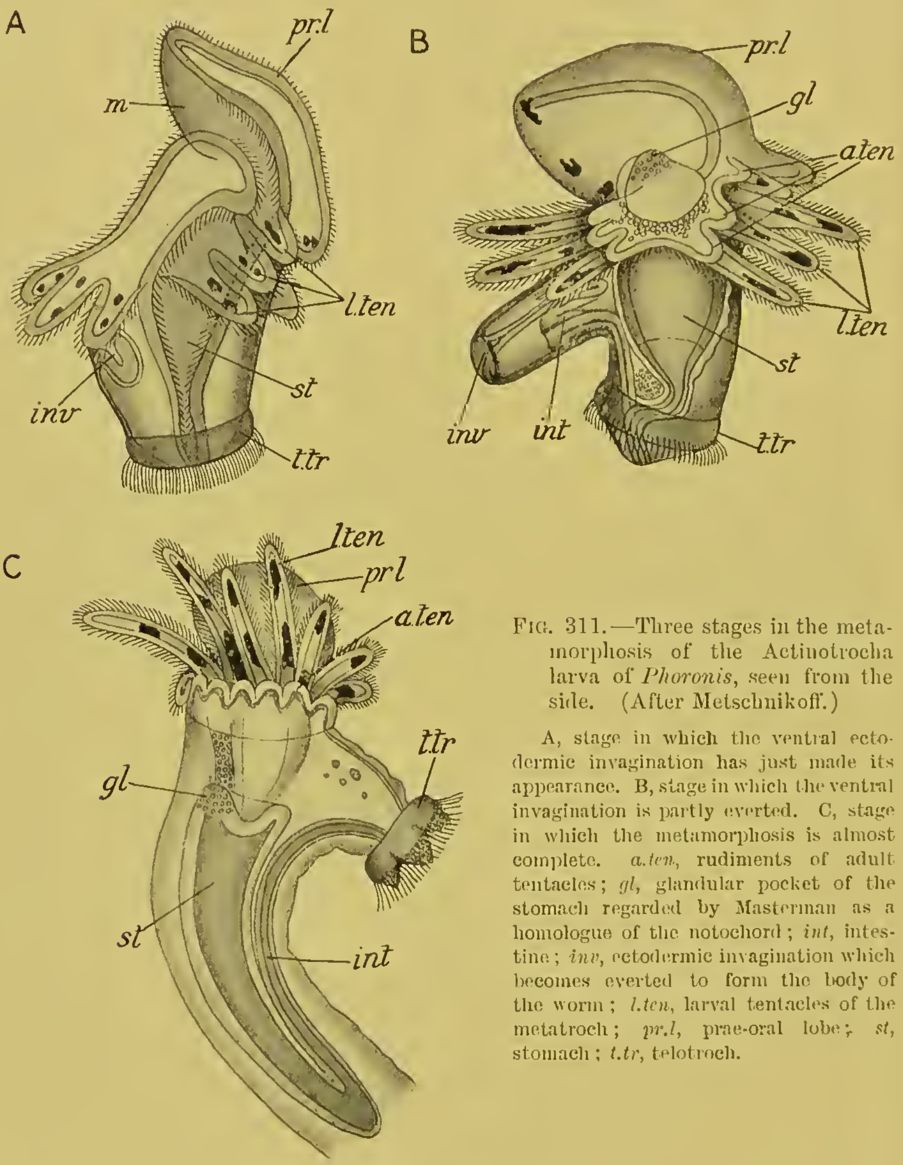

FIG. 311.—Three stages in the metamorphosis of the Actinotrocha larva of *Phoronis*, seen from the side. (After Metschnikoff.)

A, stage in which the ventral ectodermic invagination has just made its appearance. B, stage in which the ventral invagination is partly everted. C, stage in which the metamorphosis is almost complete. *a.ten*, rudiments of adult tentacles; *gl*, glandular pocket of the stomach regarded by Masterman as a homologue of the notochord; *int*, intestine; *inv*, ectodermic invagination which becomes everted to form the body of the worm; *l.ten*, larval tentacles of the metatroch; *pr.l*, prae-oral lobe; *st*, stomach; *t.tr*, telotroch.

amputated; and that this proceeding is equivalent to the casting off or absorption of the prototroch in other forms.

If this supposition be justified, then the rest of the metamorphosis can be viewed as a modification of the process of gradual growth of the ventral part of the body, already observed in *Phascolosoma* and *Sipunculus*. It is a very instructive modification, showing the kind of secondary change which may be expected to occur in ontogeny. It is another example of the complete omission of the intermediate stage of development between the Trochophore stage and the adult condition. This intermediate stage must have existed in the history of the race and doubtless occurred at one time in the history of the individual.

The general conclusion then, to which we are led by a review of the development of *Phoronis*, is that it really does belong to the group of *Sipunculus* and *Phascolosoma*, and that the classification of the older authors is so far justified. This conclusion, however, raises another series of most interesting questions. The structure of *Phoronis* is so similar to that of the Phylactolaematous Polyzoa that Lankester (1890) regarded *Phoronis* as a Polyzoan; and it seems difficult to evade this conclusion. But in that case *Phoronis* would be the only solitary Polyzoan known, and all the true Polyzoa (leaving out of account the anomalous Entoprocta) must be regarded as modified Podaxonia.

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CHAPTER XI

POLYZOA

Classification adopted—

- **Polyzoa Ectoprocta**
  - **Phylactolaemata**
  - **Gymnolaemata**
    - **Cyclostomata**
    - **Ctenostomata**
    - **Cheilostomata**
- **Polyzoa Entoprocta**

THE group of the small colonial animals known as the Polyzoa includes two divisions, known respectively as the Ectoprocta and the Entoprocta, about whose affinity with one another there is very considerable doubt. Both groups agree in being colonial, in possessing a ring of ciliated tentacles surrounding the mouth, by the action of which they obtain their food, and in having the principal nerve ganglion situated between mouth and anus on the surface which is normally turned upwards.

In the Ectoprocta the coelom is spacious and well developed, and from its walls the genital cells are developed; whilst the body is divided into a posterior part (the zooecium) and an anterior introvert (the polypide). The ring of ciliated tentacles surrounds the mouth alone, and is therefore morphologically a **metatroch.** In all these features the Polyzoa Ectoprocta resemble the Podaxonia.

In the Entoprocta, on the other hand, the coelom is entirely suppressed, except in so far as it is represented by the minute cavities of the genital organs. There are distinct nephridia, ending internally in blind ciliated ends; the body is divided into an upper cup-like part called the calyx, and a lower solid stalk. The ring of ciliated tentacles surrounds both mouth and anus, and is, morphologically, a prae-oral band or **prototroch.**

Prouho (1892), Harmer (1896), Seeliger (1906), and Czwiklitzer (1909) regard the two groups as closely allied, but Korschelt and Heider (1892) regard them as totally distinct phyla. We shall deal with them separately in this chapter, and, after having studied both, indicate our opinion as to which side in this controversy has the greater weight of evidence in its favour. We begin with the Polyzoa Ectoprocta.

POLYZOA ECTOPROCTA

In the vast majority of Polyzoa Ectoprocta the egg is fertilized whilst it is still in the maternal tissues, and undergoes the first stages of its development there. It finally emerges as a free-swimming larva, with more or less degenerate gut, and, after a short free existence, fixes itself and grows into the first person of the future colony. In a few cases, however, the eggs are shed into the sea and are fertilized there, and a comparatively long larval development ensues; this type of larva has a well-developed gut, and can feed itself. In these latter cases we have obviously the primitive type of Ectoproctan development, and it is they which deserve our closest attention. They have been most carefully studied by Prouho (1892), and we select for special description one of the forms described by him which has a long larval development.

MEMBRANIPORA PILOSA

Membranipora pilosa is a species occurring abundantly around the coasts of Europe as a delicate lace-like incrustation on the fronds of *Laminaria*. Closely allied species are found in similar situations all over the world. *Membranipora* is easily kept living in vessels of clean sea-water; the eggs are freely discharged, and develop into the young free-swimming larva, which is termed **Cyphonautes**, a name bestowed on it when it was supposed to be an independent organism. Its true nature was shown by Schneider (1869), who captured it in the sea and watched it metamorphose into *Membranipora*. To rear Cyphonautes in captivity up to this stage would require arrangements for feeding it with diatoms such as have been employed with success in the case of many other larvae.

If the vessels in which the larvae are kept have been previously coated with a layer of transparent photoxylin, then, when the larvae fix themselves, they can be removed from the sides of the vessel together with the photoxylin to which they are adherent, and cut into sections. Kupelwieser (1905), to whom we owe this method, has given us the best account of the metamorphosis of the larva. He paralysed the free-swimming larvae by adding drops of hydrochlorate of cocaine to the sea-water in which they were swimming; the larvae were then preserved in Flemming's fluid or a mixture of the solution of corrosive sublimate and glacial acetic acid.

In the development of the egg of *Membranipora* the division into blastomeres takes place in an absolutely even and regular manner, and recalls in a good many ways the segmentation of the egg of *Polygordius*. At the 16-cell stage all the blastomeres are equal to one another in size; the embryo, however, does not form a sphere, but a biconvex lens (Fig. 312), the axis joining the animal and vegetable poles being very much shortened. The **blastocoele** is excessively narrow. At the 32-cell stage the flattening is still more marked, and four blastomeres situated in the centre of one face are distinguishable by their granular contents. These blastomeres are the rudiment of the **endoderm**, and the blastula stage may be said to be now completely attained.

At the next stage the endodermal cells sink inwards, filling up the blastocoele, whilst the other cells meet beneath them, and so the **gastrula** is formed. One is thus reminded forcibly of the extreme flattening which the blastula of *Polygordius* undergoes just prior to gastrulation. The **blastopore**, or aperture left by the in-sinking of the endoderm cells, is almost immediately closed. The face on which it was situated will be called the **oral face**.

Two cells, placed symmetrically to the right and to the left of the middle line, are found at the next stage in the development of *Alcyonidium albidum*, which also gives rise to a Cyphonautes-like larva; but they have not as yet been observed in the case of *Membranipora*, though it is quite probable that they exist there also. In the case of *Alcyonidium* they help to form larval muscles, which traverse the blastocoele, and these muscles also exist in the larva of *Membranipora*. These two cells, whose exact origin Prouho could not determine, he calls the mother cells of the mesoderm. They are, however, situated in front of the mass of endoderm, and have, in all probability, nothing whatever to do with the true pole cells of the mesoderm in *Polygordius* which give rise to the coelomic wall, but are rather to be compared to the mesectoderm of *Polygordius*, *i.e.* cells derived from the second quartette of micromeres, *i.e.* from the ectoderm which gives rise to the blastocoelic muscles in the larva.

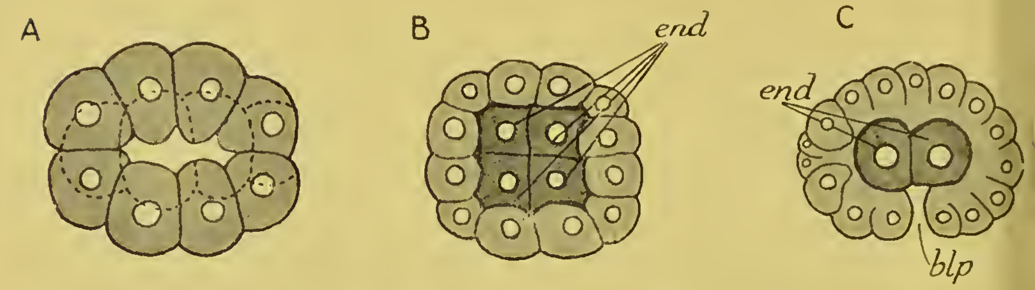

FIG. 312.—Early stages in the development of the egg of *Membranipora pilosa*. (After Prouho.)

A, stage of sixteen blastomeres seen from the side. B, stage of thirty-two blastomeres seen from the underside. C, gastrula seen from the side. *blp*, blastopore; *end*, cells which form the endoderm.

Coming now to the next changes observed in the embryo of *Membranipora*, we find that the whole embryo takes on a conical shape, the oral face forming the base of the cone, whilst at the upper end, where the point of the cone should be, a thickening of the ectoderm becomes visible, which is termed the **apical organ** and which is homologous with the **apical plate** of Annelidan and Molluscan larvae. On the oral face now appears a wide depression. This is the beginning of the enormous **stomodaeum** which pushes the endodermic mass towards the posterior end of the embryo.

The embryo now adheres by its oral face, and also by the apical organ, to the vitelline membrane, and at the same time it alters its shape, so that it becomes compressed from side to side. The vitelline membrane seems to be absorbed where it is in contact with the embryo; stiff cilia or sense-hairs appear on the apical organ, and powerful locomotor cilia on certain cells of the thickened ridge of ectoderm, termed the **mantle,** which forms the border of the oral face. The space surrounded by the mantle has become concave, and it is termed the **atrium**; into it the stomodaeum opens. The ring of ciliated cells is termed the **corona.**

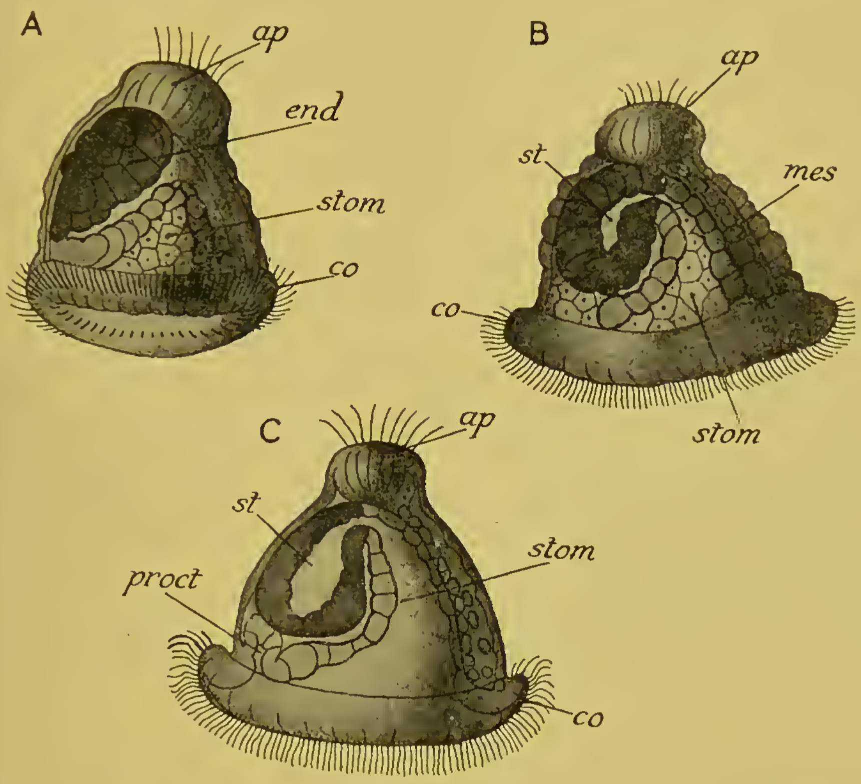

FIG. 313.—The development of the larva of *Membranipora pilosa.* (After Prouho.)

A, young larva just free from the egg-membrane; *end*, solid mass of cells which will be hollowed out to form the stomach. The stomodaeum is formed, but does not join the stomach as yet. B, young larva in which the stomodaeum has joined the stomach. C, larva a little older than that represented in B, in which the proctodaeal invagination has been formed. *ap*, apical plate; *co*, corona; *proct*, proctodaeal invagination; *mes*, ectomesoderm.

The embryo now becomes a larva and swims about; the remnants of the vitelline membrane which still envelop it in the middle are brushed off. After the free life has begun the mass of cells forming the endoderm becomes hollowed out and forms the larval stomach.

Mesoderm cells in front of this multiply and form a string leading from the aboral thickening to the ventral surface; this string is the rudiment of the main dorsal muscle of the larva. Shortly afterwards an ectodermic invagination is formed in the posterior part of the oral face. This is the **proctodaeum**, the rudiment of the **anus** and of the **larval intestine.** It grows in length and joins the stomach, and the latter opens into the stomodaeum, in which cilia become developed, and so the definite alimentary canal is completed and feeding begins. A delicate bivalve **shell** is secreted by the larva; each valve is triangular, and the apical organ protrudes between the apices of the valves, whilst their bases flank the corona.

Soon afterwards the ring of ciliated ectoderm which we have termed the corona, and which we may compare to the prototroch of the Trochophore larva, begins to exhibit modifications. In front of the mouth a pair of transverse ridges grow inwards from it at right angles to its course, and constitute a transverse band of cilia across the ventral face of the larva. A pair of similar ridges also grow inwards from the oral band in front of the anus, and constitute a second transverse band of ciliated ectoderm there.

When the Cyphonautes is fully grown it possesses two other organs: a so-called "**piriform organ**" in front of the mouth, consisting of columnar ectoderm cells, and an "**internal sac,**" which is an invagination of the ectoderm between mouth and anus. The "piriform" organ arises as an ectodermal invagination, which becomes almost shut off from the exterior, but remains connected therewith by a narrow longitudinal slit, the cells lining which are covered by powerful cilia. This slit is termed the **vibratile cleft** (*v.cl*, Fig. 314). Though these ciliated cells afterwards meet those of the corona they originate quite independently of it, and only subsequently come into contact with it. The cells of the piriform organ itself take on a glandular appearance, and emit a secretion into its cavity. The main muscle, alluded to above, leads from the apex and sides of the piriform body to the apical organ, and then passes beneath this to run down the posterior aspect of the larva to the most posterior cells of the corona.

In front of the ciliated groove which forms the opening into the piriform organ there is a rounded ciliated pit which is delimited from the groove by a blunt prominence. On the hinder aspect of this pit there is a small group of cells which carry exceptionally long cilia—cilia which, moreover, are bent in a peculiar hook-like manner, and which swing backwards and forwards in the middle line. These are termed the **vibratile plume.** A special branch of the main dorsal muscle pierces the glandular sac of the piriform organ and continues its course to end at the base of the cells carrying this special "vibratile plume." A strand of nerve fibres accompanies this muscular strand. But the main mass of the muscle and nerve proceed downwards, and whilst the nerve fibres become more numerous the muscle fibres decrease in number. Many of the nerve fibres go to the ciliated cells of the vibratile cleft, but after these have been given off the main mass of the nerve fibres proceeds to the ciliated cells of the prototroch or corona. Muscle fibres also go to the cells of the vibratile cleft,

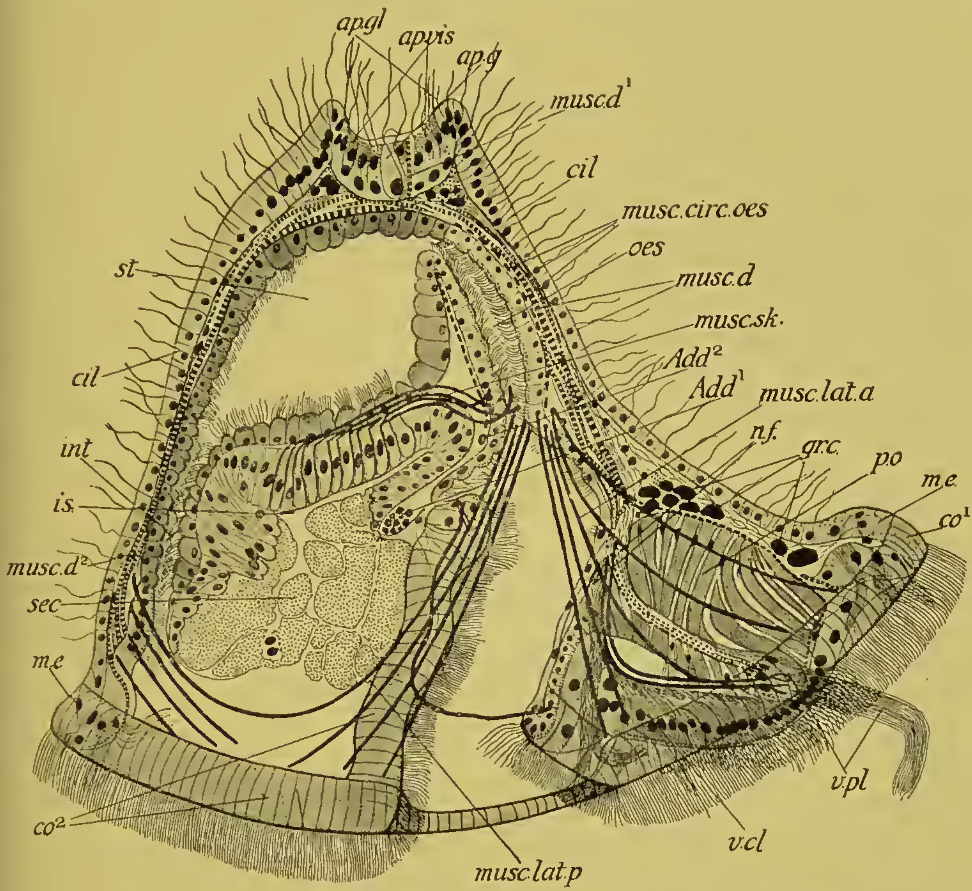

FIG. 314.—Median sagittal section of the fully grown Cyphonautes larva of *Membranipora pilosa* on the top of which have been traced certain structures (muscle fibres and corona, etc.) lying to the right of the median plane. (After Kupelwieser, slightly altered.)

Add^1, main adductor muscle of the valves; *Add*^{2}, accessory adductor muscle of the valves; *ap.g*, cells of the apical plate of the ganglionic nature; *ap.gl*, cells of the apical plate of a glandular nature; *ap.vis*, cells of the apical plate of a visual nature; *cil*, cells forming anterior and posterior borders of the larva carrying long cilia; *co*^{1}, anterior section of the corona; *co*^{2}, posterior section of the corona; *gr.c*, granular cells; *i.s*, internal sac; *int*, intestine; *m.e*, mucous cells of mantle edge; *musc.circ.oes*, circular muscles surrounding the oesophagus; *musc.d*, dorsal muscle; *musc.d*^{1}, branch of dorsal muscle which is inserted in the apical organ; *musc.d*^{2}, branch of dorsal muscle which curves back under the apical organ and is inserted in the posterior section of the corona; *musc.lat.a*, anterior part of lateral muscle; *musc.lat.p*, posterior part of lateral muscle; *musc.sk*, sucker muscle; *n.f*, nerve fibres leading from apical organ to vibratile plume and to corona; *oes*, oesophagus; *p.o*, piriform organ; *sec*, secretion produced by the cells of the internal sac; *v.cl*, vibratile cleft; *v.pl*, vibratile plume.

and to the cells of the corona, but in addition muscle fibres are given off which are inserted in the upper part of the piriform organ, and others which encircle it after the manner of circular fibres.

Kupelwieser regards the function of the piriform organ as *skeletal*, that is to say, he thinks that it affords a convenient insertion for muscle fibres; those inserted in its upper part he regards as retractors, the encircling fibres as protractors, and the vibratile plume he considers the real sense-organ. He believes that this sense-organ comes into play just before the fixed life is taken up, and that its function is to select a suitable spot for the fixation of the larva.

The **internal sac,** or **sucker,** arises just in front of the anus, and, according to Kupelwieser, it begins as a solid thickening of the ectoderm, which soon splits into two layers separated by a cavity. Prouho, however, says that it arises as an invagination just in front of the anus. This little sac develops, as larval life proceeds, into a wide, spacious sac, which is drawn out into two horns. The upper wall of the sac remains thin, but its lower wall, where it abuts on the atrial cavity, becomes glandular, and produces great masses of a slimy secretion. Eventually this wall breaks down and allows the sucker to open into the atrial cavity, into which the secretion is then discharged. Two muscles arise, one on each side, from the ectoderm of the central parts of the flat sides of the larva, and are inserted into the upper wall of the sac. These muscles, termed the **sucker muscles,** only come into play at the metamorphosis.

The ectoderm of the sides of the larva, as we have already noted, secretes two thin valves of shelly material, which thus form a **bivalve shell** protecting the larva. Round the edges of each triangular shell-bearing area there runs a cushion of large swollen cells, filled with a mucoid secretion, thus taking on the outline of a triangle. From the base of this triangle a ridge of the same material projects upwards a short distance. The ectoderm covering the narrow sides of the larva, between the valves, is covered with short cubical ciliated cells.

From this description it follows that the apical organ is bounded laterally by the cell-cushions, and front and back by cubical epithelium. The **apical organ** itself is a two-layered, slightly concave plate, or shallow cup of cells. The rim of this plate is composed of converging columnar cells, each bearing a single stiff cilium or sense-hair. Inside this outer ring comes a second ring of cells bearing pigment, to which Kupelwieser assigns a visual function, while in the centre there is a mass of clear rounded cells. From the fact that a bundle of nerve fibres proceeds from these inner cells, Kupelwieser draws the conclusion that they are of a ganglionic nature.

The fibres of the dorsal muscle penetrate between the cells of the apical organ in order to attain their insertion; but not all the fibres of the dorsal muscle, in fact only the minority, have this insertion. The majority of the fibres of the dorsal muscle pass back under the apical organ and diverge into bundles, right and left, and are inserted into the ciliated cells of the posterior part of the corona. Most of the fibres belonging to the dorsal muscle are striated, but some muscles have smooth fibres.

There is an **adductor** muscle connecting the two valves of the shell beneath the stomach, and above it there is a similar smaller muscle,

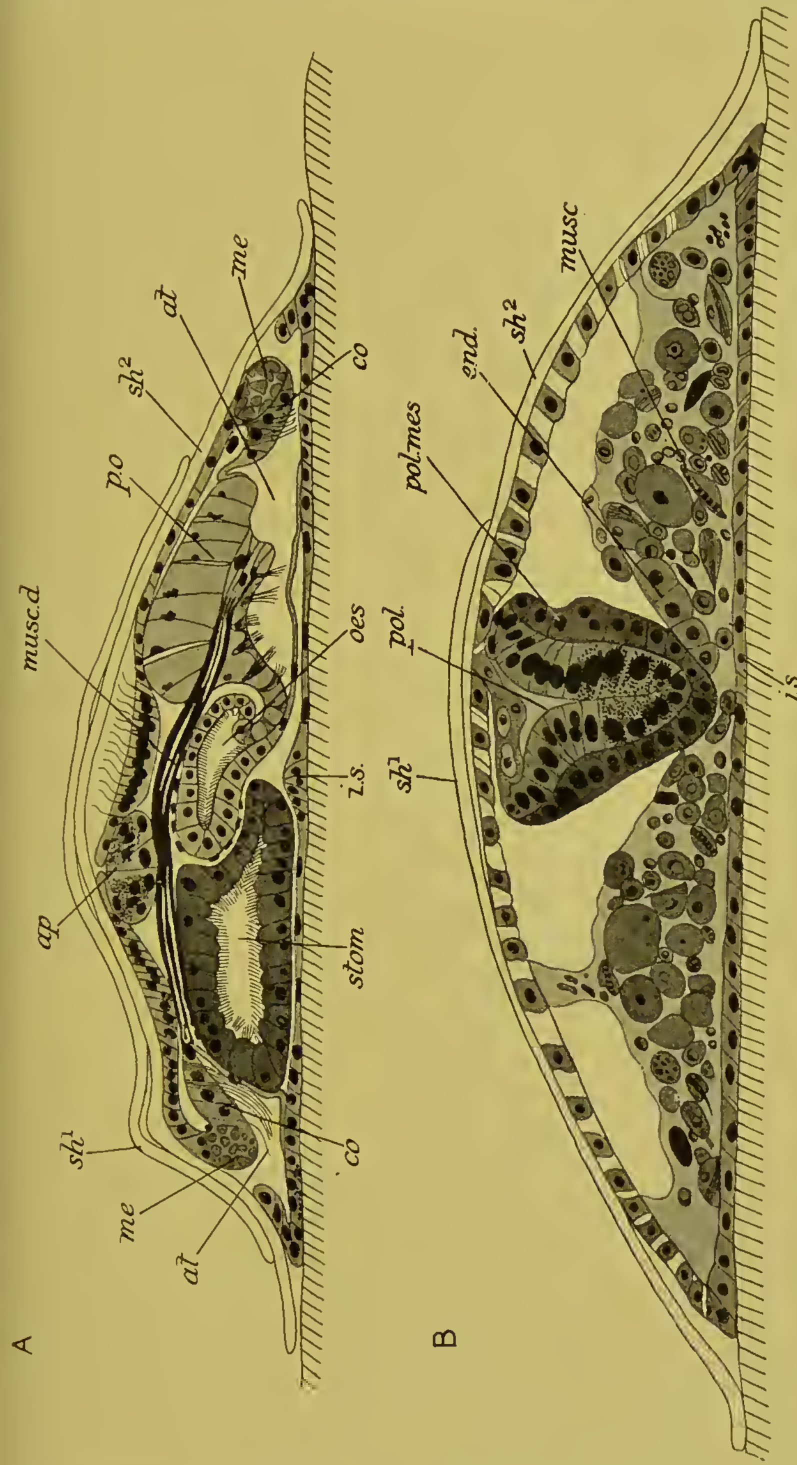

FIG. 315.—Sections through fixed and metamorphosing larvae of *Membranipora pilosa*. (After Kupelwieser.)

A, sagittal section through a larva immediately after fixation. B, frontal section through a larva in which histolysis is much advanced. *ap*, apical plate in process of invagination in order to form the polypide; *at*, atrial cavity; *co*, corona; *end*, fragment of endoderm cells from histolysed stomach; *i.s*, internal sac flattened out to form an adhesive plate; *me*, mucous cells of mantle edge; *musc*, fragment of muscle in histolysis; *musc.d*, dorsal muscle; *oes*, oesophagus; *p.o*, pyriform organ; *pol*, polypide bud; *pol.mes*, mesoderm of polypide bud; sh^1, sh^2, valves of the shell.

the **accessory adductor.** Two **lateral** muscles, an anterior and posterior, arise on each side from the same area of the shell which gives rise to the sucker muscles, and are inserted in the cells of the corona.

During its active life the larva swims with its apical organ directed forwards; but when the free life draws to an end it glides over the bottom, with its oral surface directed downwards, and during this period the vibratile plume can be seen to carry out tactile movements. Finally, the sucker is everted and forms a thin flat plate of cells which adheres to the substratum (Fig. 315). All the muscles except the sucker muscles contract strongly, and the piriform and apical organs are in consequence strongly retracted. The outer edges of the adhesive sucker turn upwards and unite with the edges of the mantle, and the remnant of the atrial cavity is converted into a ring-shaped space, towards the inner side of which the cilia are directed. Then the muscles connecting the sucker with the valves of the shell contract, and with great strength, so that the valves of the shell are, so to speak, flattened out over the compressed larva.

Histolysis of the larval tissues now begins. First the cushion cells disintegrate and their mucoid contents are cast into the ring-shaped atrium. In this way the ciliated cells of the corona become cut loose from the mantle edge, the cells of which join the edge of the adhesive plate formed from the sucker; and the remnants of the coronal cells are found floating in the ring-shaped atrial cavity. The apical organ is very deeply invaginated and broken loose from the flanking cushion cells; the adjacent ordinary ectoderm cells meet above it, and from it, afterwards, the **polypide** of the first bud, *i.e.* the alimentary canal and ciliated tentacles, are developed. The coronal cells and larval muscles are attacked by wandering amoebocytes. The stomach and intestines excrete brown granules into their respective cavities, and finally lose their cavities and become solid clumps of degenerating cells. The whole animal is thus reduced to a thin-walled sac containing, invaginated into it at one point, a thick-walled sac, which is the former apical disc and is the rudiment of the future polypide of the mother bud of the colony.

TYPES OF POLYZOAN LARVAE

Before studying the further development of the polypide it will be well to cast a brief glance at the other types of larvae which have been described in Polyzoa. All are modifications, one might add modifications in the direction of degeneracy, of the Cyphonautes type.

Prouho has indeed shown that the species *Alcyonidium albidum*, which belongs to quite a different division of Polyzoa (Ctenostomata) from that to which *Membranipora* belongs (Cheilostomata), has a larva which can be distinguished only by minute specific differences from the larva of *Membranipora*; and the same is true of the larva of *Hypophorella expansa*, which also belongs to the Ctenostomata but to a different division from that to which *Alcyonidium* belongs.

The larva of *Flustrella*, a genus allied to *Alcyonidium*, is very similar to the Cyphonautes. It has a bivalve shell, a well-developed pyriform organ, and a complete set of muscles; but the alimentary canal is somewhat degenerate, the intestine being wanting. The corona forms a complete ring without cross ridges.

In some species of *Alcyonidium*, such as *Alcyonidium polyoum*, described by Harmer (1887) (Fig. 316, A), further degeneracy can be seen; a stomodaeum and stomach alone are present, as in the larva of *Flustrella*, but the bivalve shell is gone, and the apical organ is a wide, flat disc. The corona consists of a single ring of large ciliated cells.

When we pass to Cheilostomata, like *Lepralia*, *Bugula*, etc., we find that the gut has entirely disappeared, and is represented by a mass of mesenchyme cells. The corona of *Lepralia* resembles that of *Alcyonidium*, but that of *Bugula* consists of enormously tall cells, each extending through the whole height of the larva (Fig. 316, B).

In the Cyclostomata there is also a gutless larva, but now the apical organ is represented by a deep invagination devoid of sense cells; the corona is represented by a broad belt of ciliated cells of comparatively small size, and the pyriform organ is absent.

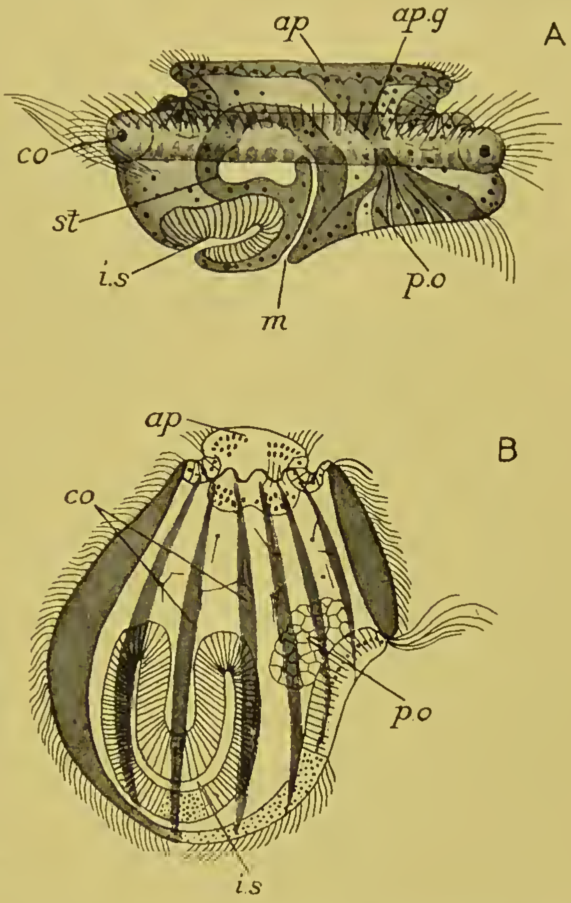

FIG. 316.—Two degenerate types of larvae of Ectoproct Polyzoa. (Combined from figures given by Korschelt and Heider.)

A, optical section of the larva of *Alcyonidium polyoum*. B, optical section of the larva of *Bugula plumosa*. The dark streaks represent coronal cells lying between the reader and the median plane of the larva. *ap*, apical disc; *ap.g*, ganglion cells beneath the apical disc; *co*, corona; *i.s*, internal sac; *m*, mouth; *p.o*, pyriform organ; *st*, stomach.

Finally in the freshwater Phylactolaemata, where, as in many other freshwater animals, there is an extremely shortened development, we find an oval larva, most of whose surface is covered with fine cilia, but which has an invagination at the anterior pole whilst the posterior pole is glandular. The broad ciliated band represents the corona, the apical invagination the apical organ, which is, however, entirely devoid of sense-hairs, and from which one or two polypides are already being developed. Fixation takes place by the posterior glandular pole, and then the walls of the anterior invagination are suddenly turned back so that the polypide area is exposed. The retroverted folds adhere to the substratum and force the larva away from its primary attachment. In this way a huge sucker-like organ is formed at the posterior pole; this sucker becomes a completely closed sac, and then its contents are devoured by amoebocytes.

It will be seen that in the series of larvae which we have just described we have to deal with a progressive disappearance of larval structures, and a progressive hurrying on of adult structures. Thus in the Phylactolaemata, which constitute the culminating point of the series, the larval body has become merely a skin enclosing the first two or three buds. It is obvious, therefore, that in seeking for light on the past history of the Polyzoan stock we must confine our attention to the primitive type of larva represented by Cyphonautes.

BUDDING

The metamorphosed Cyphonautes consists of a simple ectodermic sac with a closed vesicle of columnar cells projecting into it. This ectodermic vesicle is termed the **polypide,** and from it the ectodermic parts of the tentacles, and the whole alimentary canal of the first person of the colony, are derived. The mesodermal portions of the tentacles, including the walls of the coelomic canals which they contain, are derived from a layer of mesoderm cells (*pol.mes,* Fig. 315) which clothes the external surface of the polypide. The exact origin of these mesoderm cells from pre-existing larval mesoderm has not been determined. The ectodermic sac-like body of the metamorphosed larva constitutes the **zooecium** of the first polypide. The valves of the larval shell are soon shed and are replaced by the continuous cuticle which constitutes the ectocyst of the zooecium.

The first person of the colony originates therefore as a **bud** on the body of the metamorphosed larva; and, so far as is known, the development of this bud is quite similar to that of the later buds, by which the colony increases in size. It follows that in Polyzoa Ectoprocta, we have not the continuous life-history of an individual proceeding from the larval to the adult condition, but an **alternation of generations** by which a sexually produced form, the larva, gives rise to an asexually produced form, the first person of the colony.

The manner in which the buds of Polyzoa Ectoprocta develop has been investigated by many authors. Seeliger (1890), who investigated the buds of *Bugula,* has given the clearest account of the matter, and as his results have been confirmed in almost every point by the latest observer, Römer (1906), we shall follow Seeliger in our account.

When a new bud is about to be formed the new zooecium arises as an out-pouching of the old one. The cavity of this pouch is eventually cut off from that of the parent zooecium by a mesodermic septum, but before this happens the first rudiment of the new polypide appears in the new zooecium as an ectodermic thickening, which later becomes an ectodermic pouch open to the exterior, except in so far as it is roofed over by the common cuticle or

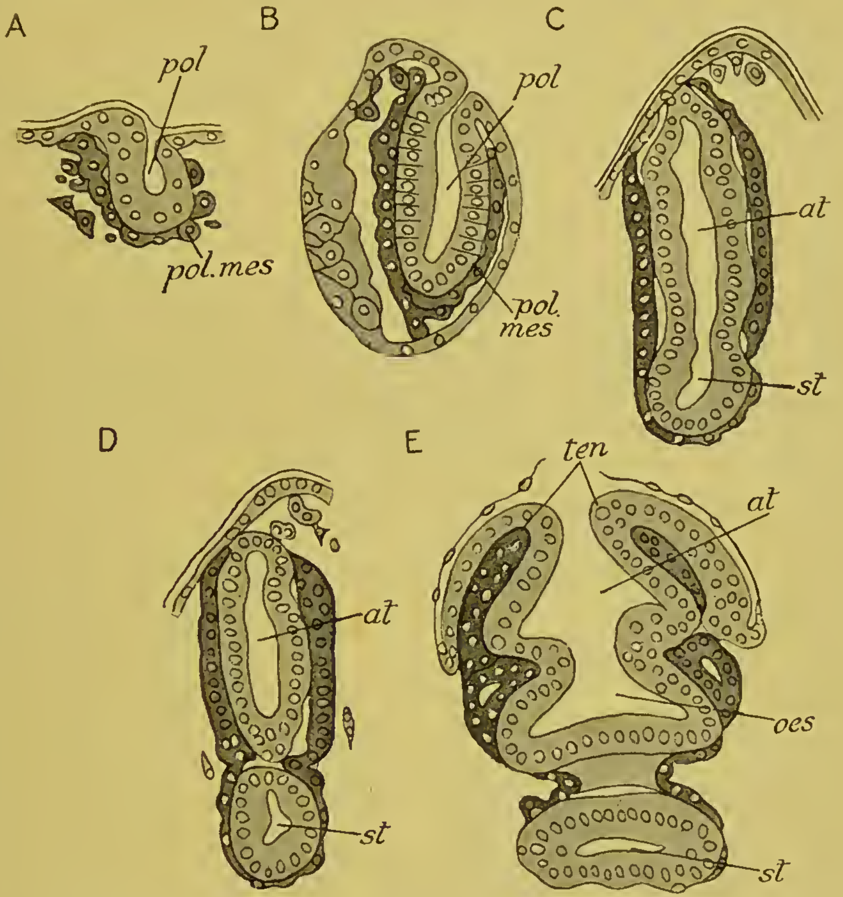

FIG. 317.—Stages in the development of the bud of *Bugula avicularia.* (After Seeliger.)

A, the early rudiment of the polypide in the form of an ectodermic invagination. B, a later stage: the rudiment of the polypide is almost shut off from the exterior, and has increased in depth, and its mesodermic covering has become continuous. C, D, two sections through an older polypide in which the constriction of the rudiment into atrium and gut has begun. C, is through the opening which remains as the anus. E, section through a still older polypide in which the tentacles have appeared as ridges in the atrial wall. The section goes through the opening which remains as mouth. *at,* rudiment of atrium; *pol.mes,* mesoderm; *pol,* polypide; *oes,* oesophagus; *ten,* tentacles.

ectocyst. This pouch deepens and its mouth closes, and the resulting sac becomes divided by a constriction into an upper region, which is the rudiment of the future **atrium** or **tentacle sheath,** with its contained tentacles, and a lower region, which is the rudiment of the entire **gut** of the new person.

The sac becomes clothed externally by mesoderm cells; these cells eventually form a coherent layer, but appear to arise as wandering cells which adhere individually to the external surface of the polypide. From this mesodermic layer the coelomic canals of the tentacles and the ring canal which unites them, are derived. The constriction between gut rudiment and atrial rudiment becomes so deep at one place as to completely sever the two from each other, but before and behind this place two openings are left by which the two rudiments still communicate, and these openings form the mouth and anus of the new person. The gut rudiment becomes divided by constrictions into **oesophagus, stomach,** and **intestine.** The atrial rudiment develops the **lophophoral tentacles** as ridges projecting into its cavity, and at the completion of development it reacquires an opening to the exterior. The retractor muscles, funiculus, etc., are derived from scattered mesoderm cells, which originate from the mesoderm cells of the mother.

Römer, who investigated the buds of *Alcyonidium*, differs only from Seeliger in finding that in *Alcyonidium* the polypide passes from the condition of a solid thickening to a closed sac at one step, and in finding mesoderm cells produced by budding from the ectoderm as well as from the maternal mesoderm cells. While this is possible we may conclude that it is unlikely.

POLYZOA ENTOPROCTA

The group of Polyzoa Entoprocta comprises only three genera, viz. *Loxosoma, Pedicellina,* and *Urnatella,* and the complete life-history has only been worked out in the case of *Pedicellina.* We shall select as type for special study *Pedicellina echinata,* the early development of which was worked out by Hatschek (1877) whose results were confirmed and extended by Lebedinsky (1905). The remarkable metamorphosis undergone by the larva was elucidated by Harmer (1887).

PEDICELLINA ECHINATA

The egg is fertilized whilst it is still in the ovary, and is penetrated by several spermatozoa, but only one unites with the nucleus while the rest are absorbed. The egg is then dehisced into the atrium of the parent, where it is retained until it has developed into a full-grown larva.

The egg segments somewhat unequally, the blastomeres at the animal pole being much smaller than those at the vegetable pole. Already when seven blastomeres have been formed a blastula stage has been reached, the upper half being formed of three small blastomeres, the lower of four larger ones; the **blastocoele** is slit-like. When, however, segmentation has been completed, there results a spherical **blastula** whose lower cells are larger and have larger yolk-granules than the upper cells. Where the border between these two kinds of cell is situated, there are to be found two cells distinctly marked off from both animal and vegetable cells, they are rounded and have unusually large nuclei which are surrounded by clear areas.

The blastula now slightly elongates so that it is bilaterally symmetrical, and its vegetative half becomes flattened, and these two peculiar cells are then situated right and left of the plane of symmetry. The cells of the vegetative half of the egg become then invaginated, and the **gastrula** stage is attained. The two large cells mentioned above are situated at the posterior end of the blastopore, but they now pass into the blastocoele and are the **mother cells of the mesoderm.** The blastopore is at first slit-like but gradually becomes restricted in length, becoming closed from behind forwards. The ectoderm, endoderm, and mesoderm are thus definitely differentiated from one another.

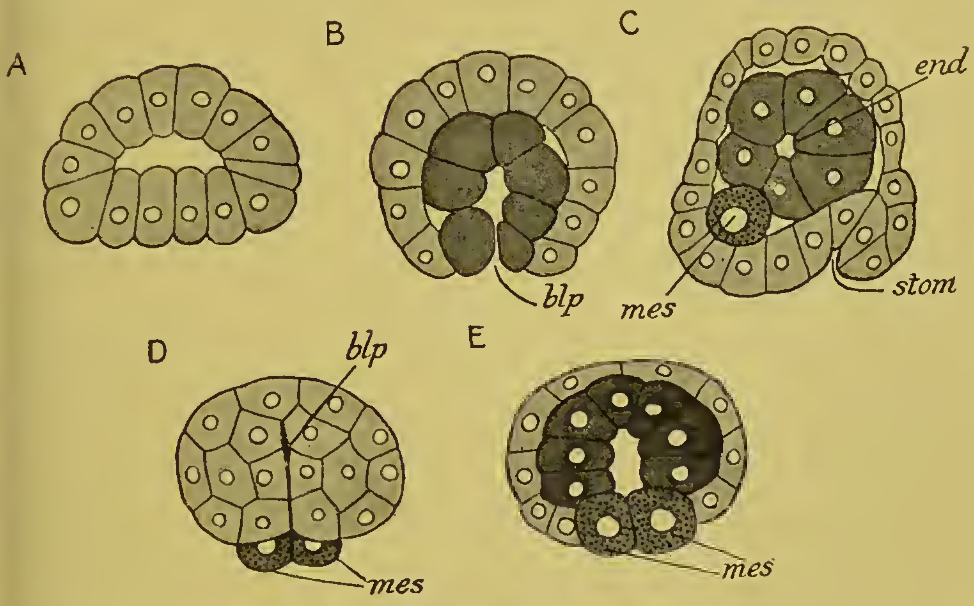

Fig. 318.—Early stages in the development of the egg of *Pedicellina echinata.* (After Hatschek.)

A, flattened blastula. B, gastrula. C, optical section of later stage nearly sagittal showing the mother cell of the mesoderm of the right side, the endodermic sac, and the stomodaeal invagination. D, view of stage in which the blastopore is closing seen from below. E, frontal section of the same stage; *blp*, blastopore; *end*, endodermic sac; *mes*, mother cells of the mesoderm.

The ectoderm flattens out except at the aboral pole. Here an **apical plate** of cylindrical cells is formed. This was termed the **cement-organ** by Hatschek, under a mistaken idea that it was the plate by which the larva fixed itself. This plate develops cilia but it soon begins to be invaginated. When the complete larval stage has been attained the walls of the invagination are still ciliated, but the cells forming the floor multiply so as to form a mass of small rounded cells several layers thick; these are ganglion cells as the development of the neuropile around them shows.

Shortly after the foundation of the apical plate, the so-called **dorsal organ** is formed on the anterior aspect of the aboral pole of the larva. It develops in exactly the same manner as the apical plate, that is, as an invaginated plate of cylindrical cells, which, however, is from the beginning markedly bilaterally symmetrical. The cavity of the invagination becomes tube-like and its walls are formed by a single layer of cubical ciliated cells, but its floor becomes converted into a bilobed ganglionic mass which is connected with the apical plate by two strands of nerve fibrils. This ganglionic mass may be compared to the brain or **cerebral ganglia** of Annelida and Mollusca, whilst the pit, which remains as dorsal organ, may be compared to the **cerebral pit** of Molluscan larvae.

Whilst these changes have been going on the blastopore has become completely closed and the archenteron separated from the ectoderm; the ventral ectoderm has become thickened, and it consists in fact of a single layer of cylindrical ciliated epithelium.

On this surface now appear three invaginations, one behind the other. The front one gives rise to the long **stomodaeum** or oesophagus, the cells lining which are all ciliated. The mouth is surrounded by specially long cilia. The stomodaeum joins the sac-like archenteron, fuses with it, and opens into it.

The hindermost invagination gives rise to the **proctodaeum**, the cells lining which carry short cilia. It is borne on a projecting portion of the ventral surface known as the **anal cone**, which, as we shall see, may be compared to the post-trochal portion of a Trochophore larva. The archenteron becomes constricted into a main sac-like portion which forms the **stomach**, on the lower wall of which glandular cells are developed, and a small bud-like **intestine** which meets and fuses with the ectodermal rectum, and so the alimentary canal is completed.

The central invagination gives rise to the **atrium** or **vestibule**, and is shallower than the others. Its outline is at first round but later becomes square. Its front wall, which abuts on the hinder wall of the oesophagus, develops into a ventral prominence known as the **epistome**; and on this is a bilobed thickening, which is connected round the oesophagus with the dorsal organ by two strands of nerve fibres, and which will develop into the main nerve ganglion of the adult.

At the sides of the atrial cavity are a pair of somewhat similar thickenings, and on its hinder wall, where it touches the rectum, is a third bilobed thickening. The lateral thickenings are obviously ganglionic in character, for they become connected with the dorsal organ, or, as we may now term it, the **cerebral ganglion**, by nervous strands. The posterior thickening is the transitory rudiment of a ganglion which disappears during embryonic life.

The edge of the atrium carries a row of specially powerful cilia which subserve locomotion and correspond to the corona of the Ectoprocta. These cilia are everted when the embryo escapes and becomes a free swimming larva; but the ridge of cells bearing them can be retracted, and then they point inwards towards the atrial cavity.

The mesoderm cells have meanwhile developed into short **mesodermal bands** on each side. According to Lebedinsky (1905) each of these bands becomes divided into three spherical somites, and in each somite a cavity appears, which we may regard as coelomic in character, just before the larva emerges from the parent vestibule. The first pair of somites become connected with the atrial cavity by long ciliated ducts which represent the excretory organs; the second pair of somites give rise to the ovaries, and the third to the testes (Fig. 319). A median **vagina** is formed by a groove-like gutter in the floor of the atrial cavity, which becomes shut off in front but remains open behind.

Besides the mesoderm that originates from the mother cells of the mesoderm, mesenchyme cells, *i.e.* wandering cells, are budded into the blastocoele from the ectoderm at two points on the posterior aspect of the aboral surface of the larva. These cells become transformed into the larval musculature and correspond to the mesectoderm of Annelida, Mollusca, and Gephyrea.

Lebedinsky's statements have been received with a good deal of scepticism. It is possible that the excretory organs do not arise as a pair of ducts connecting the coelomic cavities with the exterior, but are true nephridia; on the other hand, Hatschek saw and figured the mesodermal bands arising from the mesoderm mother cells, and it is in accordance with all that we know of other groups that these bands should give rise to the genital organs.

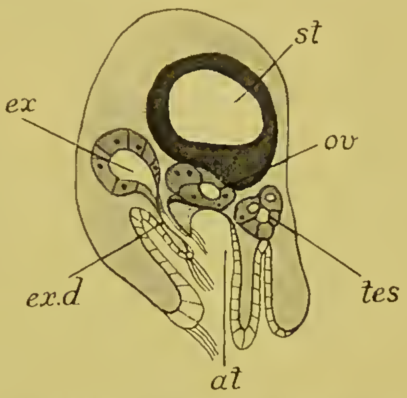

Fig. 319.—Section of the embryo of *Pedicellina echinata*, parallel to the sagittal plane but lying to the right of it. (After Lebedinsky.)

at, atrium; *ex*, excretory organ; *ex.d*, excretory duct; *ov*, ovary; *st*, stomach; *tes*, testes.

We owe the most recent descriptions of the free-swimming larva to Seeliger (1906) and Czwiklitzer (1909). Both of these authors are anxious to demonstrate the exact correspondence between this larva and Cyphonautes. Both point out that the cells forming the floor of the atrial cavity are glandular, and compare these glandular cells to the glandular cells of the "sucker" of Cyphonautes. Czwiklitzer points out further the interesting fact that the dorsal organ is only at the bottom of a ciliated pit in the retracted condition of the larva. When the larva is paralysed by adding cocaine to the water in which it swims and is then preserved, the brain is exposed on the surface and the ciliated canal is flattened out. Both Seeliger and Czwiklitzer compare the brain to the pyriform organ of Cyphonautes. The justice of this comparison will be considered later. Czwiklitzer preserved his paralysed larvae in a mixture of the solution of corrosive sublimate and glacial acetic acid, or in Fleming's fluid.

The best account of the metamorphosis of the larva we owe to Harmer (1887). He found that he could not get the larvae which

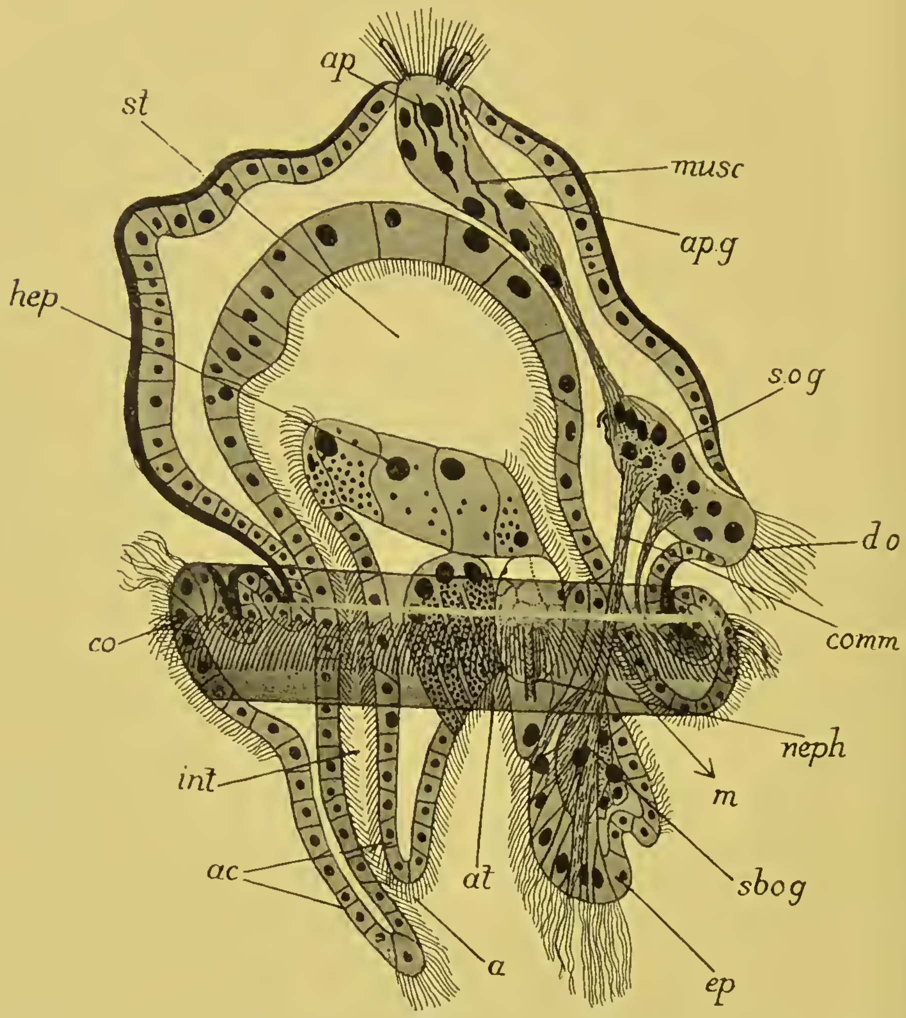

Fig. 320.—Optical sagittal section of the free-swimming larva of *Pedicellina echinata*. (After Czwiklitzer combined from several figures.)

a, anus; *a.c*, anal cone; *ap*, apical plate; *ap.g*, apical ganglion cells; *at*, atrium; *co*, corona; *comm*, nervous commissure; *d.o*, dorsal organ; *ep*, epistome; *hep*, dark granular endoderm cells constituting the so-called liver; *int*, intestine; *m*, mouth; *musc*, muscle fibres; *neph*, excretory organ; *sb.og*, sub-oesophageal ganglion; *s.o.g*, cerebral ganglion; *st*, stomach.

swarmed out from the vestibules of the parent colonies to fix themselves to the walls of the vessels in which he had placed these colonies; so he resorted to the following device. He procured colonies of *Pedicellina echinata* fixed to the calcareous seaweeds known as Corallines. He cut off all the superfluous branches of the coralline and placed the pruned stocks carrying the *Pedicellina* in vessels filled with sea-water, the mouths of which were covered with pieces of linen. The vessels were then placed in tide-pools, and were left there for one or two days. When at the expiration of this time they were examined, numerous *Pedicellina* larvae were found which had fixed themselves to the Coralline Alga. These were cut out together with small portions of the substratum to which they adhered. The Coralline Alga is softened by decalcifying it with acid alcohol, and then sections can easily be cut through it and the adhering larva.

The larva fixes itself by the edge of the atrium, which we may term the **mantle**, the corona being retracted so that its cilia point inwards. The mantle edges grow inwards along the surface of fixation so as to completely close the atrium. The anal cone no longer points downwards but, as a consequence of the greater growth of the posterior surface of the larva, points obliquely backwards. The atrium becomes divided into a lower portion near the surface of fixation, and an upper portion with which mouth and anus communicate. After the complete separation of these two portions the walls of the lower portion fold inwards, its cavity disappears, and its walls are devoured by wandering cells. The upper section is then divided into a portion near the mouth and a portion near the anus; these two portions are almost separated by the growth of the epistome and of two lateral folds which grow out from the sides of the atrial cavity.

As the metamorphosis proceeds the anal division of the vestibule grows rapidly upwards, and at the same time the retracted apical plate and brain (dorsal organ) undergo histolysis. The same fate befalls a good many of the cells lining the larval stomach, they pass into its lumen and are there reduced to structureless globules. The apex of the epistome and the tip of the anal cone likewise undergo histolysis and the cilia borne by the former are lost. When the remainder of the vestibule has rotated so far that it is directed upwards the adult ciliated tentacles begin to develop as lateral projections on its sides, and its roof is then broken through by a longitudinal slit; and so the atrium, now become the adult vestibule, is once more open to the exterior. The mouth appears to become closed by the remnant of the epistome during metamorphosis, but this flap is later perforated.

With open vestibule, developed tentacles, open mouth and anus, the young *Pedicellina* has reached the adult stage. Its stalk is formed, as will be seen if this description has been followed, from the lower part of the vestibule which has become solid. The formation of fresh buds takes place from the stalk, and the process is very similar in essentials to what occurs in the formation of buds in the Ectoprocta. A protrusion grows out from the stalk into which mesenchyme cells wander, the whole vestibule and the alimentary canal of the bud arise from an ectodermal invagination, which becomes covered with mesenchyme cells which give rise to muscles and mesodermal organs.

In endeavouring to give a phylogenetic interpretation of the developmental facts which have been narrated in this chapter it is obvious that we must commence with the life-history of the

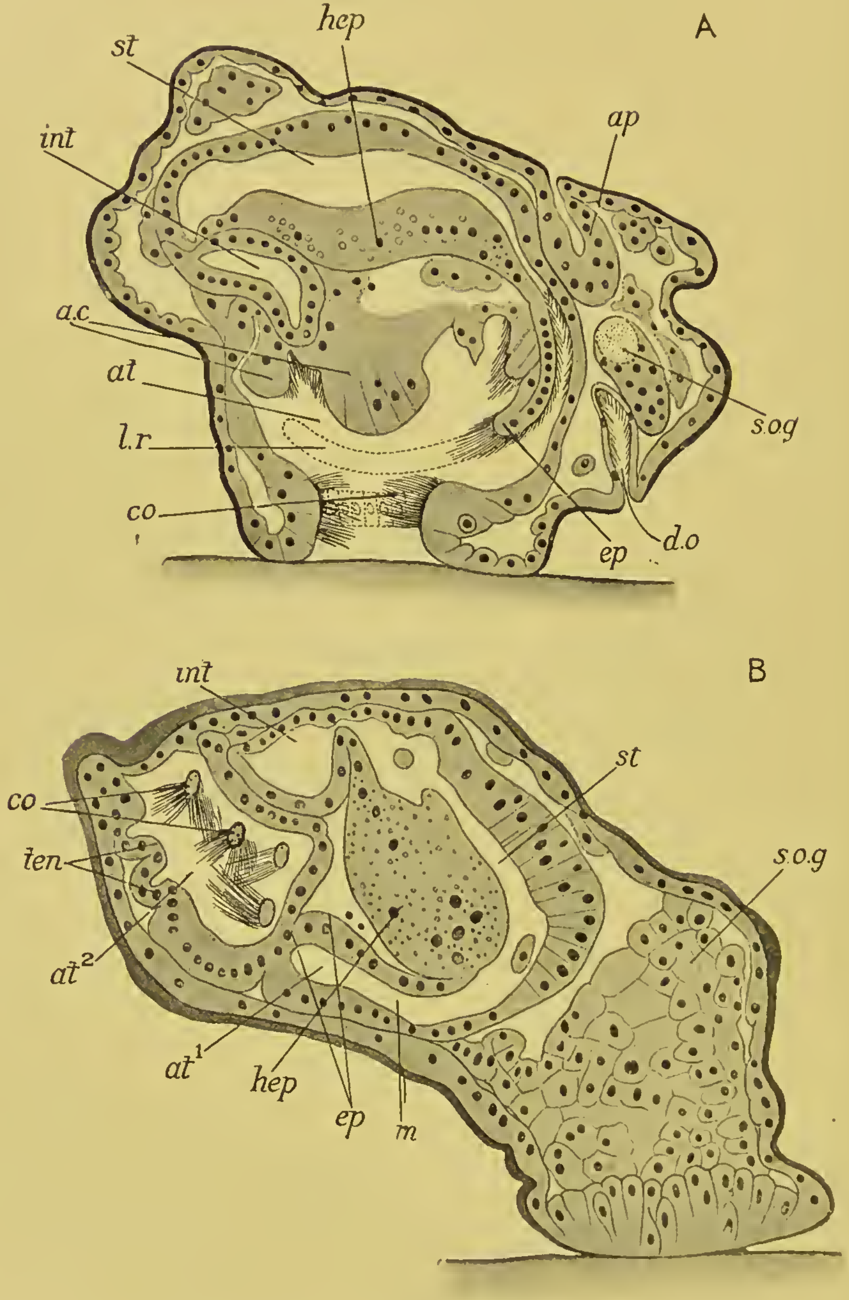

FIG. 321.—Median sagittal sections through two fixed and metamorphosing larvae of *Pedicellina echinata*. (After Harmer.)

a.c, anal cone; *ap*, invaginated apical plate; *at*, atrium; at^1, oral division of atrium; at^2, anal division of atrium; *co*, corona; co^1, degenerating fragments of ciliated cells of corona; *do*, dorsal organ, i.e. cerebral pit; *ep*, epistome; *hep*, liver cells; *int*, intestine; *lr*, lateral ridge which later, with epistome, divides the atrium into two halves; *m*, position of the mouth; *s.o.g*, brain (supra-oesophageal ganglion; *st*, stomach; *ten*, rudiments of tentacles of the adult.

Entoprocta, because only in the case of this group are the larval organs taken over into the adult.

Now the larva of the Entoprocta, as all investigators admit, is closely related to the Trochophore type of larva. The apical plate is identical in both forms, the corona obviously corresponds to the prototroch, whilst the anal cone of the Entoproct larva may be compared to the post-trochal region of the Trochophore.

If Lebedinsky is to be trusted (and Hatschek confirms him in some points) the Entoproct larva in reality corresponds to a post-trochophoral stage in Annelid development, since the mesodermal band already exhibits traces of division into three somites. On the other hand the evanescent posterior and lateral ganglia, discovered by Lebedinsky, suggest a comparison with the pleural and visceral ganglia of Mollusca, and we have already seen reason to imagine that primitive Mollusca, when they diverged from the Annelidan stock, may have had incipient segmentation represented by two or three somites. The "dorsal organ" may be compared to the cerebral ganglia of the Annelida and Mollusca, which are found a little distance from the apical plate itself.

But starting from an ancestor still retaining the habit of a Trochophore though possessing a segmented mesoderm, how are we to interpret the metamorphosis? The closing of the vestibule or atrium and the consequent shutting off for a time of both mouth and anus from the exterior must be a secondary feature, for, during the whole history of the race, mouth and anus must have been continuously functional. Therefore, although it is correct to say that in individual ontogeny the larva fixes itself by the whole of its ventral surface, yet this fixation must represent, in the history of the race, a prae-oral attachment.

Our Entoprocta, therefore, would be exceedingly primitive Annelida or exceedingly primitive Mollusca, which had become attached just in front of the mouth, and which have, like Cirripedia and Crinoidea, undergone such extensive growth of the region of attachment as to rotate the mouth upwards into a more advantageous position for catching floating prey.

The Ectoproct larva in its primitive form, as exemplified by Cyphonautes, is also allied to the Trochophore larva; apical plate and corona have obviously the same significance as in the Entoproct larva. But the Cyphonautes represents the Trochophore in an earlier stage of development than does the Entoproct larva, since it possesses no true mesoderm derived from pole cells, and has no secondary cerebral ganglion.

The attempt to compare the pyriform organ to the dorsal organ of the Entoprocta, although boldly essayed by Seeliger, must be pronounced a failure. The pyriform organ, as Kupelwieser has demonstrated, is not a nerve ganglion at all but a peculiar sense organ, and its position within the circle of the corona cannot be compared to that of the dorsal organ of the Entoprocta, which is outside the circle of the corona.

The fixing organ of the Cyphonautes larva is the eversible sucker, and this is a modification of the ventral surface *between mouth and anus*, and corresponding to the region which, in *Phoronis*, is everted to form the worm-like body of the adult, and the region which grows into the long worm-like body of the Sipunculoidea. This latter group, as we have seen, are certainly, and *Phoronis* is probably, derived from a Trochophore stock.

We regard, therefore, the Polyzoa Ectoprocta as a degenerate group, allied to *Phoronis* and the Sipunculoidea, which have become fixed by the ventral surface and have learned how to bud. It follows that Entoprocta and Ectoprocta, though both derived from the Trochophore stock, in common with all Annelida and Mollusca, have acquired a fixed life in different ways and at different periods; and are not descended from a common fixed Polyzoan ancestor, but are two separate offshoots from the Trochophoran stock. It follows also that Polyzoa Ectoprocta alone can be regarded as having affinities with Podaxonia, and that Polyzoa Entoprocta cannot be included in this group, as defined by Lankester (1890).

The fact that in the formation of buds the value of the germinal layer is quite altered, and that tentacles and atrial wall, along with the alimentary canal, arise from a common rudiment, will be considered when the similar phenomena amongst Tunicata (Urochorda) are discussed.

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CHAPTER XII

BRACHIOPODA

Classification adopted—

1. **Ecardines.** 2. **Testicardines.**

The group of the Brachiopoda is, by some zoologists, regarded as being allied to the Polyzoa Ectoprocta. Like the Ectoprocta, the Brachiopoda are fixed by a peduncle or foot which may be regarded as a ventral protrusion from the body. Like them too they possess a **lophophore**, *i.e.* a lip surrounding the mouth from which arises ciliated tentacles. They possess a pair of trumpet-shaped execretory organs which also serve as genital ducts, and are therefore **coelomiducts**, not true nephridia, and the nerve centres remain throughout life in connection with the ectoderm.

Brachiopoda might be regarded as an offshoot from the Podaxonia, and this view is adopted by Lankester (1890). The study of their development therefore becomes a matter of great interest.

Of only four forms, all belonging to the division Testicardines, is the complete life-history known; these are *Argiope neapolitana*, *Cistella neapolitana*, *Thecidium mediterraneum* and *Terebratulina septentrionalis*. The development of the first two has been worked out by Kowalevsky (1874), but he did not employ modern methods; his researches were carried out a long time ago. The development of *Thecidium mediterraneum* was described by Lacaze-Duthiers in 1869, and only the external features of the development were noted. The eggs in all these three species pass through the earlier stages of their development in brood pouches, and only escape as larvae at an advanced stage of development. But in the case of *Terebratulina septentrionalis* they are shed into the sea, they adhere for a little time to the chaetae of the mother, and escape as larvae at an early period in development. Of the development of this species we possess two accounts, one describing the external features of the entire development, by Morse (1873), and the other giving the results of the application of modern methods to the embryonic and free-swimming larval stages, by Conklin (1902). This form, therefore, will be selected for special description.

TEREBRATULINA SEPTENTRIONALIS

Conklin did not himself collect and preserve the eggs and embryos; these were collected for him and preserved in Perenyi's fluid by Dr. Gardiner. Advantage seems to have been taken of the spontaneous spawning of males and females when brought into the laboratory. According to Morse, the egg, when laid, is slightly kidney-shaped and about $\frac{1}{6}$th millimeter long, and, as seen by Conklin in the preserved state, is oval.

The segmentation of the egg differs widely from that of any Trochophore larva so far studied. It is true that it divides into two slightly unequal blastomeres, then by a furrow at right angles to this, into four, and then by a circumferential furrow into eight blastomeres. It is true also that the "cross furrow," characteristic of spiral cleavage, is sometimes seen in the 4-cell stage. But the type of cleavage is not constant, and it finally results in the formation of a **blastula** in which there are a large number of cells absolutely indistinguishable from one another, and in which it is impossible to discriminate an animal from a vegetable pole. Sometimes in the earlier stages of cleavage four larger cells are observed to be budding off smaller ones, but the final result is always exactly the same whatever the mode of cleavage; in all cases a hollow spherical blastula is formed which is ciliated all over.

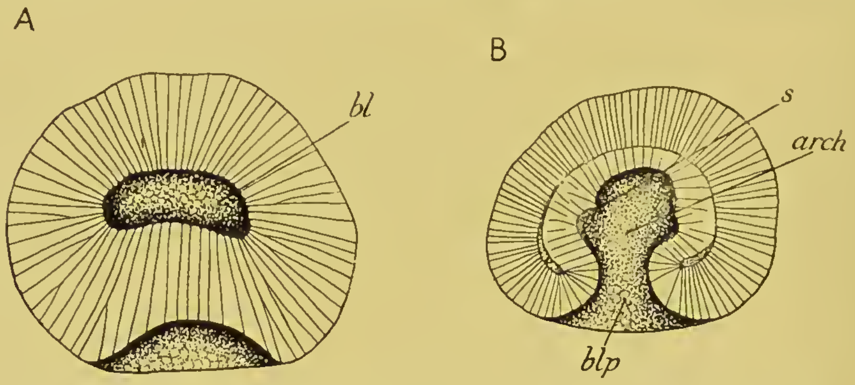

FIG. 322.—Optical sections of early embryos of *Terebratulina septentrionalis*. (After Conklin.)

A, blastula, invagination of the vegetative pole just beginning. B, gastrula, invagination complete: beginning of ridge which divides the archenteron into enteron and coelom. *Arch*, archenteron; *bl*, blastocoele; *blp*, blastopore; *s*, shelf which delimits enteron from coelom.

Although no experiments have been made to determine the point, it seems fairly clear that, in the segmentation of the Brachiopod egg, the cell divisions do not, as in Annelida and Mollusca, separate organ-forming substances one from another, but that we have to do with **indeterminate cleavage.** We shall become closely acquainted with this form of cleavage when we study the eggs of Echinodermata.

The cells of which the blastula consist are long and cylindrical and carry long cilia. The cilia are inserted in basal granules of unusually large size. The blastula becomes converted into a **gastrula** by a wide-mouthed invagination. The invaginated cells as they pass inwards change their form and become cubical, but of course still retain their cilia. This change of form is the first histological differentiation observed in development. The **archenteron** is very spacious and fills up almost the entire interior of the gastrula, so that the segmentation cavity or **blastocoele** is reduced to a narrow slit. The embryo has now a somewhat conical shape, since, at the place opposite to the blastopore, it is pointed.

The next change which occurs is the division of the archenteron into **gut** and **coelom** (Fig. 322). This takes place by the outgrowth of a crescentic shelf of cells one layer thick, arising from what afterwards is seen to be the anterior side of the archenteron. This shelf arises from a position fairly high up on the wall of the archenteron, and grows backwards and downwards into its cavity. It is bilaterally symmetrical, *i.e.* it is equally developed on the right and left sides. By this outgrowth the archenteron is divided into an oval gut above, and a broad flat coelom below, which underlies the enteron and overlaps it at the sides. The two chambers, however, still open into each other posteriorly, and the lower one communicates with the exterior through the **blastopore.**

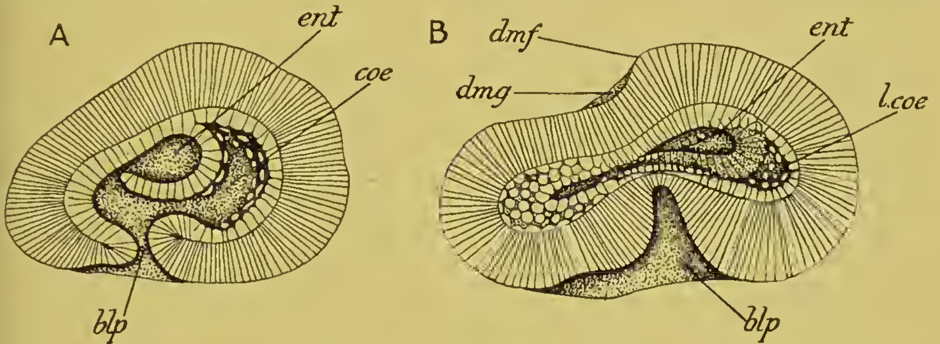

FIG. 323.—Later embryos of *Terebratulina septentrionalis* seen from the side—in optical section. (After Conklin.)

A, stage in which the enteron is nearly delimited from the coelom, but in which the blastopore is still open. B, stage in which the blastopore has been closed, leaving a pit in its place, and in which the hinder part of the archenteron has been obliterated. *blp*, blastopore; *coe*, coelom; *d.m.f*, dorsal mantle-fold; *d.m.g*, dorsal mantle-groove; *ent*, enteron; *l.coe*, left lobe of coelom seen from behind the enteron.

The next change which occurs is that the embryo becomes flattened in a dorso-ventral direction, and the coelom becomes divided into right and left portions in the middle of its course, owing to the gut being, as it were, pressed through it into contact with the ventral ectoderm (Fig. 323).

At the same time the blastopore becomes closed, and the manner in which this takes place is important. It becomes changed in shape from a large round opening to a narrow slit-like groove. Then the edges of this groove approach one another and finally cohere in the posterior part of the embryo. In the anterior portion of the groove a pore opening into the archenteron persists for some time. Finally this is closed, but a shallow pit is left, and in this same spot, at a later date, the invagination to form the **stomodaeum** arises. In this way a valuable landmark is created for the correlation of the region of the larva with reference to the adult organs.

On the opposite side of the larva from that on which the stomodaeum is situated—a side which we must regard as the dorsal side—a depression appears in the form of a transverse groove which is the beginning of the **mantle-groove**, and the embryo now takes on the form of a top with a broad anterior and a narrow posterior region. At the same time the communication between the gut and the coelom becomes completely closed.

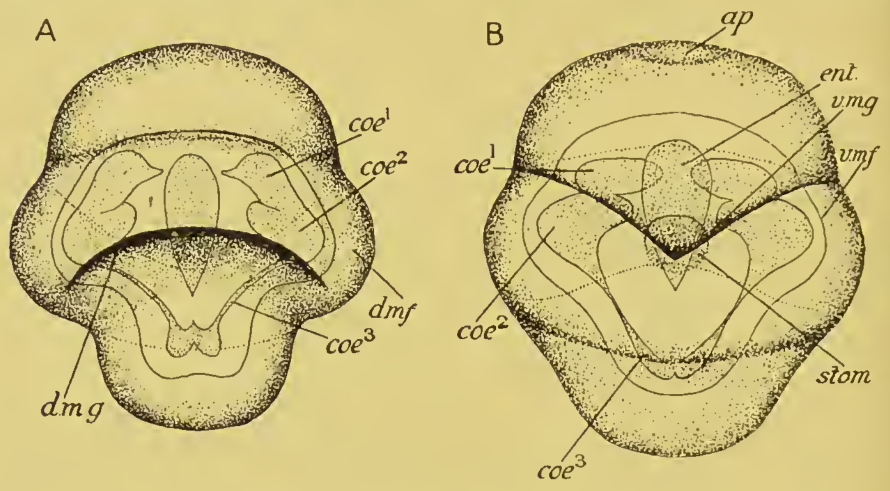

FIG. 324.—Dorsal and ventral views of a larva of *Terebratulina septentrionalis*. (After Conklin.)

A, dorsal view. B, ventral view. *ap*, apical plate; *coe*^{1}, *coe*^{2}, *coe*^{3}, the three divisions of the coelom on the left side; *d.m.f*, dorsal mantle-fold; *d.m.g*, dorsal posterior mantle-groove; *ent*, gut; *stom*, rudiment of the stomodaeum; *v.m.f*, ventral mantle-fold; *v.m.g*, ventral anterior mantle-groove.

In the dorsal region the **mantle-fold** appears as a fold or lip overhanging the mantle-groove in front. This fold has a crescentic form, being situated farthest forward in the middle portion, and inclining downwards and backwards at the two sides. It soon extends on to the ventral surface in the form of two backwardly directed folds which meet in the mid-ventral line at an acute angle. The point where they meet is situated just behind the spot where the last trace of the blastopore was seen, and where the pit is found which marks the site of the future **stomodaeum** (Fig. 324).

When the mantle-fold has been completed, it will be observed that there is a mantle-groove not only behind it but also in front of it. The **anterior mantle-groove** marks off a "**head segment**," whilst the part of the larva behind the **posterior mantle-groove** is the "**foot segment**," the middle region being known as the "**mantle segment.**" The posterior groove is deepest on the dorsal side, the anterior groove on the ventral one (Fig. 325). The mantle-fold grows rapidly backwards until it almost completely covers the foot segment. At four places on the edge of the mantle, two near the mid-dorsal line and two more laterally situated, invaginations are formed, and the cells at the bottom of these sacs give rise to long chaetae. These chaetae were not observed by Conklin, but the sacs were visible in his sections, the chaetae had dropped out.

Whilst these changes have been taking place the coelom has been undergoing development. When we last referred to it, it consisted of right and left sacs which communicated with one another in front and behind. These communications, however, become closed by the proliferation of cells into the cavity of the coelom. The coelomic cavities become narrow, almost vestigial, in the region of the foot, but in the head region they expand and take on a trefoil form. In the mantle region the coelom sends out dorsal and ventral extensions into the mantle-folds. This section of the coelom alone remains wide and spacious; the cavities of the head and foot coelom almost disappear in consequence of the thickening of their lateral walls. When viewed from the side, the coelom is seen to be regularly divided into head-, mantle, and foot lobes, and there is a specially narrow part in the middle of the foot behind which it widens out again. This last dilatation is regarded by Shipley (1883) as forming a fourth segment (Fig. 325).

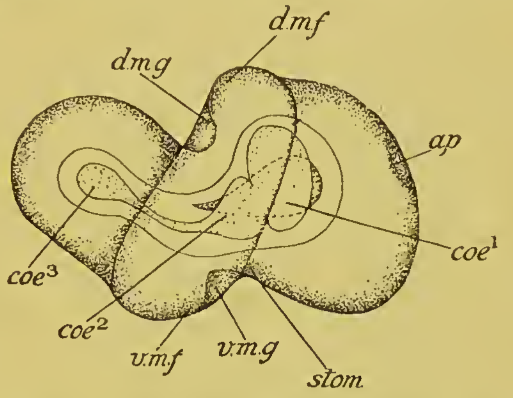

FIG. 325.—Lateral view of a larva of *Terebratulina septentrionalis*. (After Conklin.)

Letters as in preceding figure.

The gut is a flask-shaped sac, the broad end of which is anterior and the narrow end posterior. In the latest stage which Conklin examined, a ventral outgrowth of endoderm cells is formed, extending towards the pit already referred to, which marks the site of the last trace of the blastopore. There is no doubt that this is the rudiment of the endodermal part of the oesophagus.

Just in front of the dorsal apex of the larva the ectoderm is slightly invaginated. The invaginated cells are very long and slender and carry a specially long tuft of cilia (Figs. 326 and 327). At their bases small rounded ganglion cells are cut off, which, however, remain in close contact with the ectoderm. The plate so formed is evidently homologous with the **apical plate** of the Trochophore larva, and the cells at its base are the rudiment of a **supra-**

oesophageal ganglion. A quite similar sense-plate is formed in the mid-ventral line behind the rudiment of the stomodaeum. Whether this plate bore cilia or not was not determined, but its cells are the same shape as those of the apical plate, and, from their bases, ganglion cells are cut off which are the rudiment of the important **sub-oesophageal ganglion** of the adult (Fig. 326).

Before this stage has been reached the embryo has become transformed into a free-swimming larva of most characteristic form. The body is divided by constrictions into three segments. Of these the anterior, or head segment, is conical and ciliated all over like the hood of the Actinotrocha larva, but carries in addition an apical tuft of long cilia; the second, or mantle segment, carries two long backwardly directed folds, a dorsal and a ventral, both of which are ciliated; the dorsal carries the bundles of chaetae on its edge; the third, or foot segment, carries no cilia or chaetae. Since the larva possesses no mouth, and is therefore unable to feed itself, its free life must be of short duration.

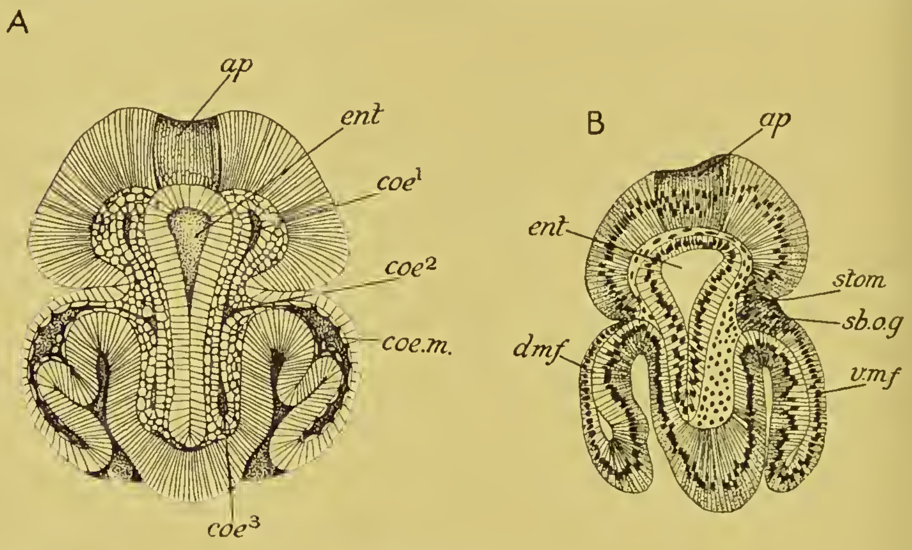

FIG. 326.—Frontal and sagittal sections of the larva of *Terebratulina septentrionalis*. (After Conklin.)

Letters as in the two preceding figures. In addition, *coe.m*, extension of coelom into mantle-fold; *sb.o.g*, rudiment of suboesophageal ganglion.

For the account of its metamorphosis we are entirely indebted to Morse, and, as he did not use the method of sections, we know only the external features of this period of the development of the larva. After swimming for some time, not in any case longer than twenty-four hours the larva fixes itself to a suitable substratum by the end of the foot. This "foot" becomes transformed into the peduncle or stalk of the adult. Then the mantle-folds lose their ciliated covering, first on the ventral and then on the dorsal side (Fig. 327); then they are suddenly retroverted so as to project forwards instead of backwards, and so hide, not the foot, but the head segment. On the outer surface of the mantle-folds the valves of the **shell** now make their appearance. The first chaetae are very long; they are of a provisional nature and are soon shed, and the sacs containing them disappear, but in their place there appear more numerous chaeta-sacs with short chaetae, which persist through life. The head segment becomes less and less prominent. The first ciliated tentacles of the **lophophore** make their appearance at the sides of the head segment (Fig. 328). Others subsequently appear so as to form a transversely oval ring surrounding the mouth. A part of this ring extends up on to the base of the dorsal mantle-lobe, but there is no doubt that the whole of the lophophore must be regarded as belonging to the head segment; later the oval ring becomes produced at the corners which constitute the adult "**arms.**" The pores in the shell, so characteristic of Brachiopoda, are already to be seen. These are caused by the outgrowth of blind tubes from the mantle coelom through the ectoderm (*coe.c*, Fig. 329).

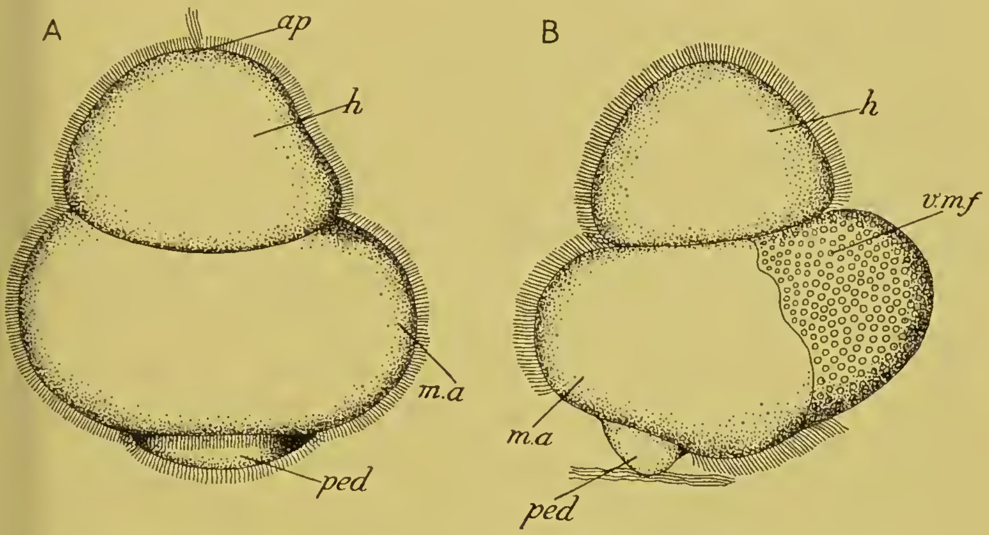

FIG. 327.—Two larvae of *Terebratulina septentrionalis* just before and at the time of fixation. (After Morse.)

A, free-swimming larva. B, larva at the moment of fixation. *ap*, apical tuft of cilia; *h*, head segment; *ma*, mantle segment; *ped*, foot segment; *v.m.f*, area of the mantle segment which is about to become the ventral mantle-fold and has lost its cilia.

AFFINITIES OF THE BRACHIOPODA

It must be obvious to the reader that there is still a wide and promising field for further investigation in the elucidation of the organogeny of a Brachiopod. Conklin's investigations, which have done more to clear up the subject than any other, were done, as we have seen, on material collected and preserved for him, as an interlude in his more serious work on Molluscan and Tunicate Embryology.

If some investigator were to devote a summer to the subject and to repeat Morse's observations on the living larvae, if better methods of preservation were used and the celloidin-paraffin method of embedding employed, as described in Chapter II., there is no doubt that a flood of light would be thrown on the subject. Professor Morse himself, who has deserted zoology for other fields of research, once expressed to us his regret that no one had so far followed in his footsteps, and he maintained that the larvae could be reared through their metamorphosis without any difficulty.

Whilst, however, awaiting the advent of a zoologist who will thoroughly clear up the subject, a few provisional conclusions may be briefly indicated. Professor Morse himself entertained no doubt that the Brachiopoda were an ancient offshoot from the Annelidan stem, basing his conclusion on the presence of distinct segments and of chaeta-sacs in the larva.

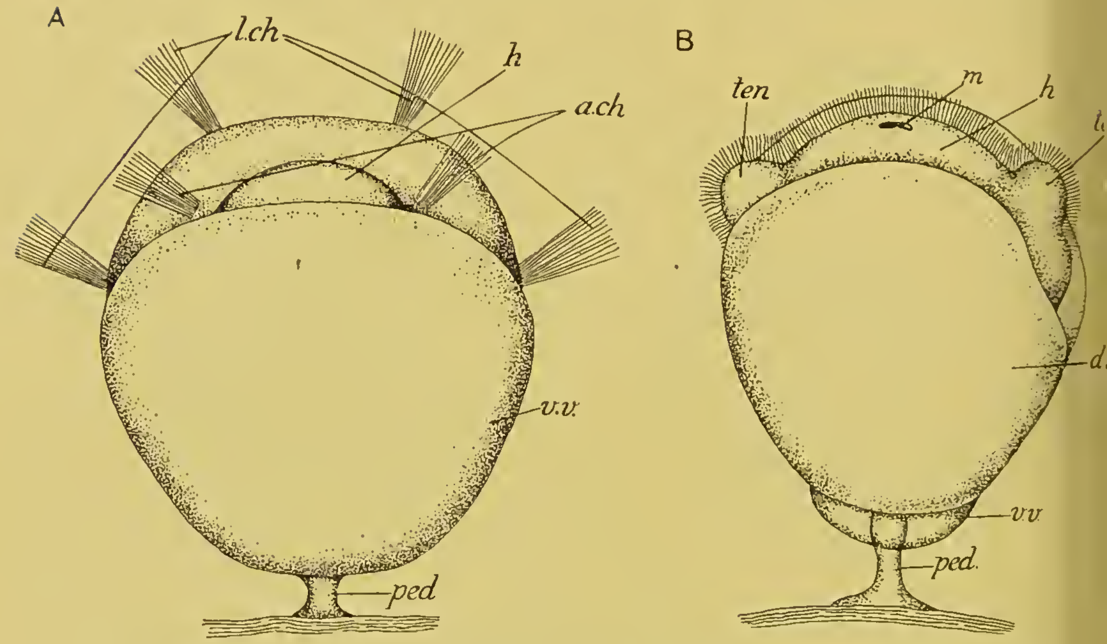

Fig. 328.—Two young *Terebratulina septentrionalis* immediately after the metamorphoses. (After Morse.)

A, specimen showing six bundles of provisional chaetae. B, specimen showing the origin of the first tentacles. *a.ch*, adult chaetae; *h*, head segment; *d.v*, dorsal valve; *l.ch*, larval chaetae; *m*, mouth; *ten*, first tentacles of the lophophore; *v.v*, ventral valve; *ped*, peduncle.

In most Brachiopoda there is no anus at all, although there is a blind and apparently functionless intestine; but in a few archaic genera, grouped together as Ecardines, an anus exists. In *Lingula* this is situated on one side, near the origin of the peduncle—which in this genus alone is muscular,—in the groove between dorsal and ventral mantle-lobes. In *Crania* it is situated in the middle line, at the posterior end of the animal, since in this genus the foot segment is absent and attachment is effected by the whole surface of one valve.

It is obvious, therefore, that the foot segment cannot be regarded as a true segment in the Annelidan sense of the word. It is probably to be regarded as a ventral protrusion of the body, similar to the protrusion which forms the greater part of the body in Podaxonia, with this difference, that in recent Brachiopoda, at any rate, it remains solid and no part of the alimentary canal passes into it.

But we have already reached the conclusion that the Podaxonia represent an early offshoot from the Annelidan stem, and, if the Brachiopoda are to be regarded as showing affinities with the Podaxonia, Morse's general conclusion would be justified in a roundabout manner.

Conklin's researches have added certain points in favour of Morse's view. The existence of an apical plate with a tuft of long cilia, and above all the position of the mouth as ventral and posterior to the top-shaped ciliated head segment, which may be regarded as representing—as it does in Podaxonia—an enlarged prototroch, are facts which seem to point to an essential identity of structure between the Brachiopod and Trochophore larvae.

On the other hand, the origin of the coelom as a division of the archenteron, and the indeterminate cleavage, are points in which the development of the Brachiopod diverges widely from that of any Trochophore larva so far studied, unless Erlanger's results with regard to the development of *Paludina* should be finally sustained. They recall forcibly the development of Echinodermata.

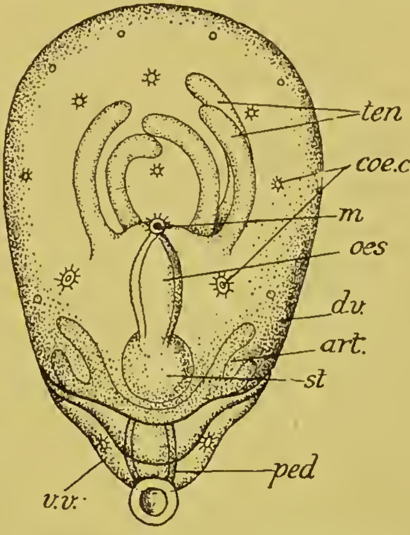

Fig. 329.—Young *Terebratulina septentrionalis* some little time after metamorphosis, viewed from the dorsal surface. (After Morse.)

art, articulating processes of valves—a notch on each side on dorsal valve; *coe.c*, blind extensions of mantle-coelom perforating the shell; *d.v*, dorsal valve; *m*, mouth; *oes*, oesophagus; *ped*, stalk; *st*, stomach; *ten*, tentacles of lophophore; *v.v*, ventral valve.

We shall reserve a final conclusion on the affinities of the Echinodermata and Brachiopoda until the development of the former group has been studied, but we may remark, in the meantime, that the coelom might be described as a hollow, bilobed but unpaired, ventral diverticulum of the gut.

If we view it in this light we shall be forcibly reminded of Erlanger's still unrefuted statement as to the origin of the coelom in *Paludina*. We had already arrived at the conclusion that, in the Trochophore, the origin of the coelomic mesoderm from a single large mother cell (4d), which was originally situated in the gut wall, must be interpreted as a reminiscence of pouch-formation, which had been suppressed because the number of embryonic cells present at that stage had been diminished.

This consideration brings us to the final point. **Determinate cleavage,** in which each cell division separates specific organ-forming substances, is distinguished from **indeterminate cleavage,** in which the individual cell is a subdivision of no importance, by the fact that in the former type there are fewer and larger blastomeres present at any given stage than in the latter.

Now the Brachiopod has almost certainly got indeterminate cleavage of the egg. It would be natural, therefore, to expect that Brachiopoda should retain the pouch-method of forming the coelom. That the coelom, according to Kowalevsky, is cut off as a pair of lateral pouches in *Argiope,* and as a single-bilobed pouch in *Terebratulina,* does not seem to be of any particular importance, for quite similar variations in the method of coelom formation are met with amongst Echinodermata.

If we imagine that at one time the Ctenophore-like ancestor of Annelida possessed indeterminate cleavage, and that the change to determinate cleavage is a later specialization, then we might imagine that the Brachiopoda were an offshoot from the Proto-annelidan stem before determinate cleavage had been acquired.

If this conclusion were justified, the Brachiopoda could not be included amongst the Podaxonia, but would represent an analogous offshoot occurring at a much earlier date; and thus Professor Morse's characterization of the Brachiopoda as "ancient cephalized worms," contrasted with Gephyrea, Serpulids, etc., as "modern cephalized worms," would be found to contain a large measure of truth.

Great light on many obscure questions of Brachiopodan embryology would be obtained by a successful elucidation of the development of *Lingula,* a genus which has persisted almost unchanged since early Cambrian times.

The late Professor Brooks (1878), of Johns Hopkins University, Baltimore, found a free-swimming larva of this form and described its metamorphosis. This larva, however, represents a much later stage in development than the free-swimming larvae of other Brachiopoda, so far studied, because the mantle-lobes are already retroverted, and the valves of the shell and the first rudiments of the lophophore are formed. In fact it bears much the same relationship to the larva of *Terebratulina* as the Molluscan veliger larva does to the Molluscan trochophore. It is to be hoped that some one of the many brilliant pupils of Professor Brooks may take up their master's work and carry it to a successful conclusion.

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CHAPTER XIII

ROTIFERA

Classification adopted—

1. **Floscularidea.**	4. **Asplanchnidea.**
2. **Melicertidea.**	5. **Scirtopoda.**
3. **Bdelloida.**	6. **Ploima.**

THE Rotifera are a puzzling group of minute animals about whose affinities most contrary views have been held. The extremely small size of the adult, and consequently of the egg, has made the investigation of the development extremely difficult, but within comparatively recent times Zelinka (1892) has published a most satisfactory account of the development of *Callidina russeola*, and this we shall accordingly select as type. Jennings (1895) traced the cell-lineage of the egg of *Asplanchna Herricki* for a few generations, but, as he did not correlate the regions of the cellular embryo to the organs of the adult which resulted therefrom, his work is of no value for us.

CALLIDINA RUSSEOLA

Callidina russeola belongs to the division of Rotifera known as Bdelloida, in which there is an eversible proboscis in the mid-dorsal line, and in which the ciliated organ is represented by two semicircular retractile hoops placed at the sides of the proboscis, and in which the body ends in a "foot" terminating in a pair of forceps. *Callidina* lays what is, for its size, a comparatively large egg. The egg-case measures ·1 mm. in length, and the whole of the development takes place after laying; a period of seventeen days is required before the completed embryo escapes from the egg-case. *Callidina* is an inhabitant of the drops of water which cling to the thickly matted leaves of moss plants, and the eggs can be found by rinsing the moss in water and examining the washed-out rubbish under a low power. It is, however, better to catch adult specimens of *Callidina* and keep them in clean watch-glasses until they lay their eggs.

These eggs have a somewhat sticky exterior and are apt to become coated with particles of dust, etc. If the water in which they lie is kept clean and changed, they will develop perfectly normally until the perfect Rotifer hatches out. Most of Zelinka's observations seem to have been made on the living egg. The egg and its surrounding case are of an oval shape, and at one end there is a fine circular line in the shell, the plane of which is at right angles to the long axis of the egg. This marks the spot where the end of the egg-case will come off, as it does, in a lid-like manner, and where the embryo will emerge.

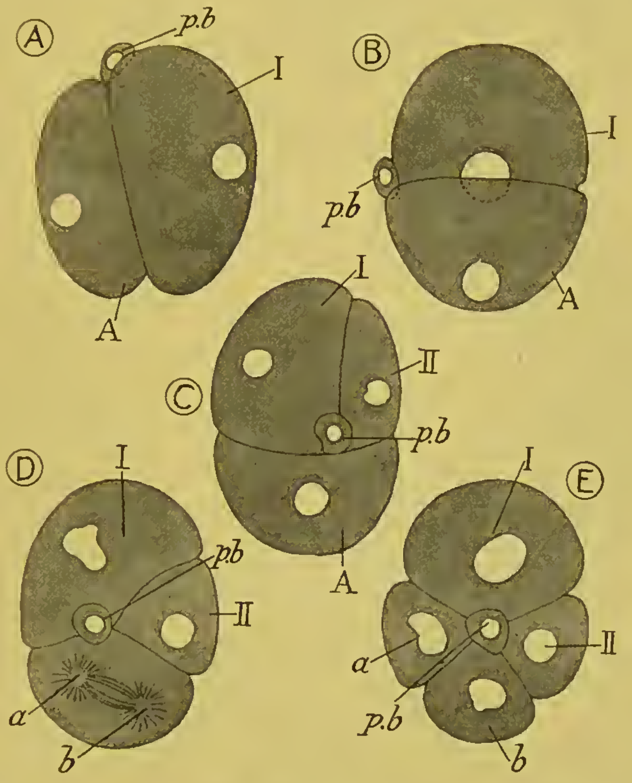

Fig. 330.—Early stages in the segmentation of the egg of *Callidina russeola*. (After Zelinka.)

A, 2-cell stage from the side, before rotation. B, 2-cell stage from the side, after rotation. C, D, 3-cell stage and preparation for 4-cell stage, from dorsal surface. E, 4-cell stage, from the dorsal surface. *p.b*, polar body.

The development is parthenogenetic: no male has yet been found in the case of *Callidina*. Five hours after the egg is laid a single polar body of comparatively large size is given off from the upper pole. It does not remain here, however, but rotates, along with the general contents of the egg, on to a surface which eventually turns out to be the dorsal surface of the embryo (Fig. 330, B).

Before this has happened the egg-nucleus has retreated from the spot where the polar body was given off, to the centre of the egg.

Here the first spindle is formed, almost transverse to the long axis of the egg, and the first cleavage plane is consequently longitudinal and divides the egg into two cells, a larger, denominated I, and a smaller one called A, lying side by side. The polar body lies at one end of this furrow, which, since it is near the pole where the polar body was given off, we shall call the animal or upper pole of the egg.

With the rotation, however, of the polar body, which has just been described, the cleavage plane separating the first two blastomeres also rotates, so that from a position almost parallel to the long axis of the egg it takes up a position almost transverse to this axis. In consequence of this rotation the smaller cell A swings round until it occupies the posterior pole of the egg. Next a small cell is cut off by a longitudinal cleavage plane from the right side of the larger cell. This cell is denominated II. It slips down along the right side to the posterior pole of the egg. Then A divides into two cells, *a* and *b*; *b* comes to occupy the posterior pole of the egg, whilst *a* and II come to occupy opposite sides of the egg. In this way a characteristic lozenge-shaped 4-cell stage is formed (Fig. 330).

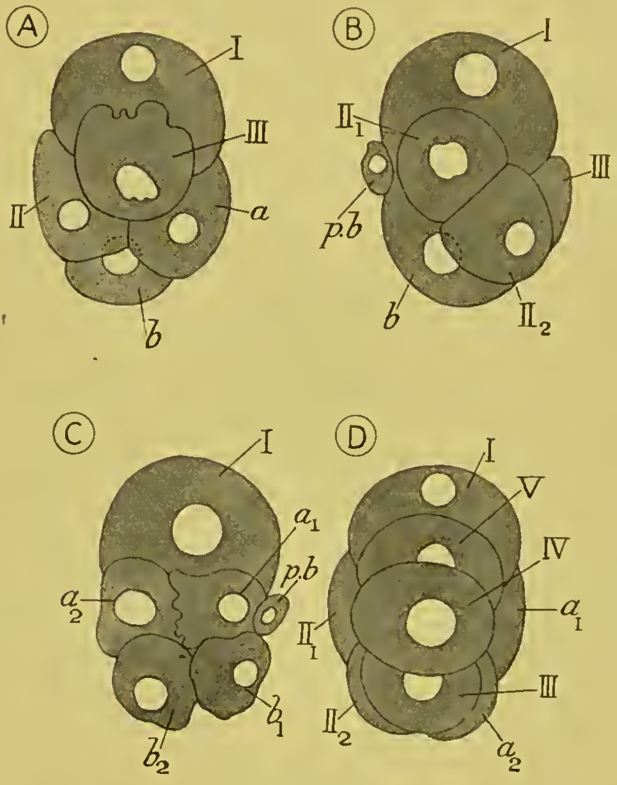

FIG. 331.—Further stages in the segmentation of the egg of *Callidina russeola*. (After Zelinka.)

A, 5-cell stage seen from the ventral surface. B, 6-cell stage seen from the right side. C, 9-cell stage seen from the left side. D, 10-cell stage seen from the ventral surface.

After a pause a smaller cell III is given off from the large cell I on its ventral side; this cell then slides back towards the posterior end and pushes the cell *b*, which till now has occupied that position, on to the dorsal surface. Then II divides into II_1 and II_2 by an oblique cleavage-plane; but this plane rotates till it is transverse and II_1 is in front of II_2. About an hour later *a* divides into a_1 and a_2, and *b* into b_1 and b_2, by planes of division parallel to the long axis of the egg. Then I gives off, just as it did before, a cell on the ventral side. This is denominated IV, and it slides back in the same way as did III to the posterior end of the egg; b_2 and b_1 are now both dorsal, and a_1 has rotated till it is in front of a_2, and b_1 is in front of b_2 (Fig. 331).

In this 9-cell stage, therefore, the large cell I occupies the anterior pole of the egg, and behind it are four longitudinal rows of two cells each, making up the rest of the egg, thus $\begin{array}{c}\mathrm{I}\\ a_1\ \mathrm{IV}\ \mathrm{II}_1\ b^1\\ a_2\ \mathrm{III}\ \mathrm{II}_2\ b_2\end{array}$. The large cell I gives off still another small cell V on the ventral side, which passes backwards, pushing III back so that it occupies the posterior pole of the egg. Then the three lateral and dorsal rows of cells, *i.e.* $II_1 - II_2$, $a_1 - a_2$, and $b_1 - b_2$, divide by cleavage-planes, transverse to the long axis of the egg, into rows of four cells each.

Soon III divides into III_1 in front, and III_2 behind, and this raises the ventral row —V, IV, III_1 III_2—to the number of four cells. The large cell I and the three uppermost cells of the three dorsal rows, viz. a_1, b_1, II_1, are distinguished from the rest by the greater abundance of yolk granules in them.

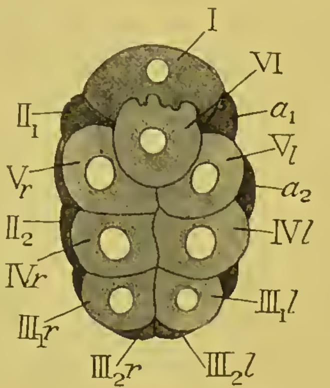

Fig. 332.—Sixteen-cell stage in the segmentation of the egg of *Callidina russeola*. (After Zelinka.)

Where a cell has divided into right and left sisters these are marked with the suffixes *r* and *l*.

The next change is the doubling of the ventral row by cleavage-planes parallel to the long axis of the egg. We have now five longitudinal rows of four cells each, two of these being ventral and three dorsal.

But soon the dorsal rows begin to be doubled in the very same manner; the granular cells, however, usually remain undivided, but occasionally become divided into right and left sisters as shown in Fig. 333, while the cell I gives off, for the last time, a cell VI on the ventral side; the latter slides back in the usual manner and presses the daughters of III_1 and III_2 on to the dorsal surface. The effect of this is to shove forward the capping cells of the dorsal rows, which, as we have already seen, are distinguished from the rows to which they belong by being more granular. Thus, pushed forward, this crown of three granular cells begins to overarch the front end of the large cell I.

The **blastula** stage may now be said to be attained. It consists of the large cell I, of six dorsal rows of three cells each, capped by three granular cells, of two ventral rows of four cells, and of an odd cell VI in the mid-ventral line.

Gastrulation now begins by the retiring of the cell I from the surface. This cell may now be denominated E, as it is the mother cell of the endoderm. This cell may, however, before its inward movement begins, be divided into front and posterior cells. Whether this has occurred or not it speedily does divide in this way, and then by longitudinal planes into right and left halves, and then by further transverse planes, till a mass of eight cells has been produced. Then the three granular cells, if they have not divided before, now divide each into two cells lying side by side, and the two larger rows of ventral cells are also subdivided by longitudinal planes giving rise to four rows. The six granular cells become compressed into a mass and are no longer arranged in a crescent form (Fig. 333).

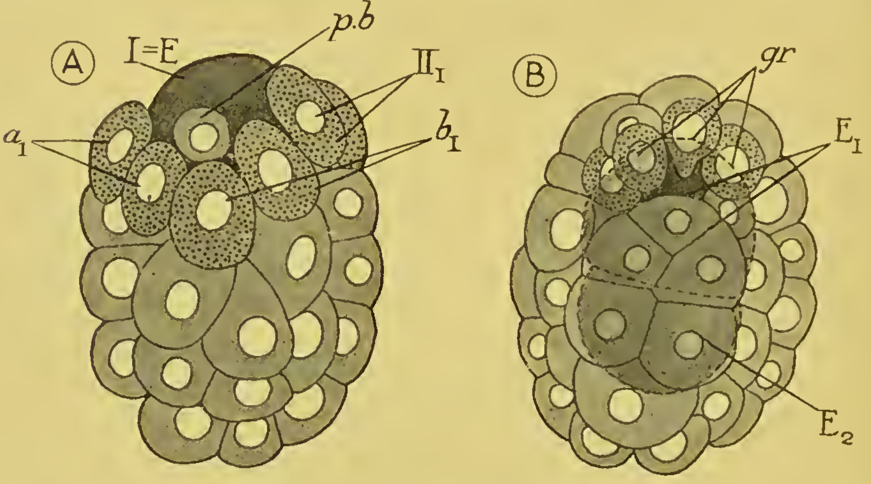

FIG. 333.—Two views of embryos of *Callidina russeola* showing the process of gastrulation. (After Zelinka.)

A, dorsal view of an embryo in which the endoderm cell (I=E) is just sinking inwards. In this embryo the granular cells a_1, b_1, and II_1 have each divided into right and left sister cells. This is exceptional. This division usually does not occur until somewhat later. B, ventral view of a somewhat later stage in the process of gastrulation. The granular cells have passed round the anterior end of the embryo on to the ventral surface. The endoderm cell has divided into anterior (E_1) and posterior halves (E_2), and is almost completely enclosed by ectoderm. *gr*, granular cells; *p.b*, polar body.

This mass is the rudiment of what we may term the **primary stomodaeum.** It becomes forced from its anterior position on to the ventral surface by the backward pressure of the cells on the ventral surface—for these ventral cells continue their initial tendency to push backwards and to pass on to the dorsal surface at the posterior pole of the egg; thus they force forward the rows of dorsal cells, and it is this pressure which forces the anterior granular cells downwards round the front end. To reach this stage requires twenty-four hours from the time that the egg is laid.

The next change which occurs is the invagination of the rudiment of the **primary stomodaeum**. The layer of clear ectoderm cells now forms a complete mantle all round the egg. The granular cells divide and form a two-layered plate of cells lying obliquely across the egg. Soon afterwards the outer clear ectoderm, at the spot where the granular cells were covered over, is invaginated in the form of a wide funnel, which is the **secondary stomodaeum** (Fig. 334).

Soon afterwards the whole ventral surface becomes concave, and in this way the rudiment of the **tail**, which occupies the hindermost part of the concavity, is marked off from the rest of the body. The front border of the concavity, which is at the same time the hinder border of the mouth, is the rudiment of the **under lip**. The tail, once begun, grows rapidly forwards, so that the embryo is bent double (Fig. 334).

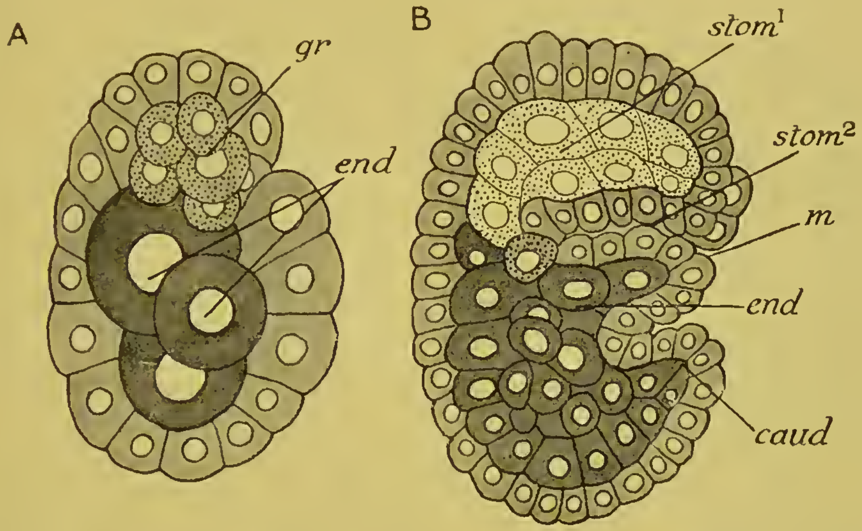

Fig. 334.—Optical sagittal sections of two embryos of *Callidina russeola* after the process of gastrulation has been completed. (After Zelinka.)

A, earlier stage. B, later stage: the secondary stomodaeum is formed and the differentiation between head and tail has begun. *caud*, rudiment of the tail; *end*, endodermal cells; *gr*, granular cells; *m*, mouth; *stom*^{1}, primary stomodaeum consisting of the granular cells; *stom*^{2}, secondary stomodaeum consisting of ordinary ectoderm.

In front of the mouth an overhanging lobe of cells becomes prominent; this is the rudiment of the **apical plate** out of which both proboscis and trochal plate grow. It is kidney-shaped in outline, its longest axis being transverse to that of the embryo, and its concave border being in front. The concavity deepens until the plate is obviously bilobed (Fig. 335).

Meanwhile the endoderm cells have been undergoing division, and they become grouped into two masses, the front one being derived from the anterior four cells and the hinder from the posterior four. The posterior mass of cells is contained in the tail or foot, whilst on the anterior mass rest, directly, the mass of granular cells forming the still solid rudiment of the primary stomodaeum. From the sides of the anterior mass two solid outgrowths are developed, which are the rudiments of the **genital organs**, *i.e.* of **vitellarium** and **germarium**; for in Rotifers, as in Platyhelminthes, a portion of the genital rudiment consists of rudimentary ova, which serve as food to the few ova capable of development, and is termed the vitellarium.

The ectoderm cells in the dorsal region, just behind the "apical plate," multiply rapidly and project inwards as a solid invagination. This is the first rudiment of the **brain**. One day later a second, larger invagination of the ectoderm takes place in the centre of the apical plate. It is nearly solid, but a slight hollow appears at the surface which soon flattens out. This second ectodermal invagination is the second rudiment of the brain; it impinges on the first, which covers it as a kind of sheath (Fig. 336).

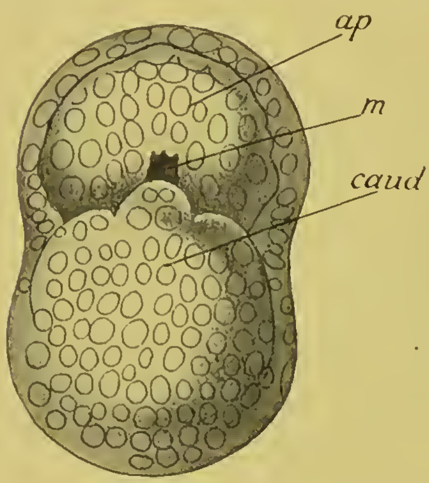

Fig. 335.—Ventral view of embryo of *Callidina russeola* somewhat older than those represented in Fig. 334. (After Zelinka.)

ap, rudiment of apical plate; *caud*, rudiment of tail; *m*, mouth.

Soon after, the inner stomodaeal rudiment assumes the form of an oval body, in the centre of which a fine slit-like cavity appears, which is in direct communication with the secondary stomodaeum and mouth. The primary stomodaeum is now seen to be the rudiment of the peculiar pharynx termed the **mastax**, characteristic of the Rotifers. Some portions, however, of this stomodaeal rudiment remain over after the formation of the pharynx, and at a later stage give rise to the **salivary glands**. The cavity in the pharynx grows wide, and it is curved in such a way as to leave a large median hump on its floor. The hump becomes covered with a thick cuticle, and this cuticle is the first indication of the **trophi** or jaws.

The head region now begins to grow rapidly. Indeed, the development might be said to be characterized by an alternate growth of the head and foot. The growth takes place in the middle of the apical plate and forms an elevation which is the rudiment of the **proboscis**. The remainder of the apical plate flattens out and forms a shelf around this elevation. On this shelf at a later period the **trochal** cilia are developed, and the place where they will arise is now indicated by two semicircular strips of cuticle. This shelf is therefore the rudiment of the **trochal disc** (Fig. 337, A).

The cells forming the front endodermal rudiment lose their outlines and form a syncytium; they then develop a cavity in their interior which is the cavity of the mid-gut. About the same time, on the dorsal side of the foot, a hollow invagination of ectoderm appears, this is the **proctodaeum**, that is the rudiment of the anus and hind-gut.

We have already seen that the hinder endodermal rudiment is contained in the foot, but before the anus is formed it begins to be displaced by a solid terminal invagination of ectoderm, which is the rudiment of the **foot gland.**

In the following period of development the head region undergoes complicated developments. The proboscis is bent ventrally. Between it and the cuticular strips, mentioned above, a solid invagination of ectoderm appears on each side of the trochal disc. Then the whole head region is retracted and surrounded by an upgrowth of the ventral lip, which, however, does not quite reach the dorsal side. Later this upgrowth develops the outer cilia known as the **cingulum.** Still later, however, the whole head region—proboscis, and apical shelf, is again protruded; and the cuticular strips are specially thickened at each side, where the shelf shows an indentation.

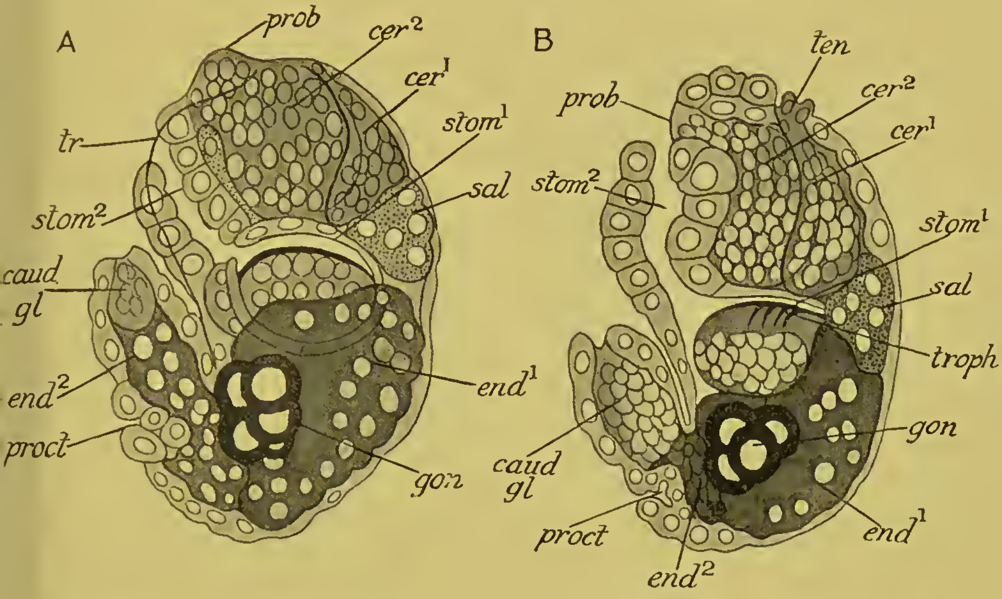

FIG. 336.—Optical sagittal sections through two late embryos of *Callidina russeola* in order to show the formation of organs. (After Zelinka.)

A, stage of predominant growth of the tail. B, later stage: predominant growth of the head region. *caud.gl*, foot gland; *cer*^{1}, *cer*^{2}, first and second rudiments of the cerebral ganglion; *end*^{1}, endodermic rudiment of the gut; *end*^{2}, endodermic rudiment of the excretory bladder; *gon*, genital rudiment; *prob*, rudiment of proboscis: *proct*, proctodaeum; *sal*, salivary gland; *stom*^{1}, inner stomodaeum or mastax; *stom*^{2}, outer stomodaeum; *ten*, tentacle; *tr*, border of region of apical plate which gives rise to the "wheels"; *troph*, rudiment of one of the trophi or jaws.

The ectodermal thickenings between these and the proboscis are protruded as hemispherical bosses. Subsequently these are again invaginated, and remain so till the embryo is born, when they are finally protruded. They are then surrounded with cilia, developed from the regions of the cuticular bands, and form the "**wheel-organs**" of the adult (Fig. 337, B). They eventually become connected with each other by a very narrow upper lip, which runs above the mouth and beneath the proboscis in such a manner that the latter is excluded from the trochal field. The dorsal feeler, or **antenna**, is formed at the spot where the first rudiment of the brain still retains a connection with the ectoderm.

The muscles all appear to be of ectodermal origin. Those first observed by Zelinka were longitudinal fibres running from the "wheel-organs" to the middle of the body. Circular muscles are developed later from ectoderm cells, at regular intervals; these cells retain their plasma and their individuality, in contradistinction to most of the other ectoderm cells which flow together and form a thin syncytium, the so-called "hypodermis."

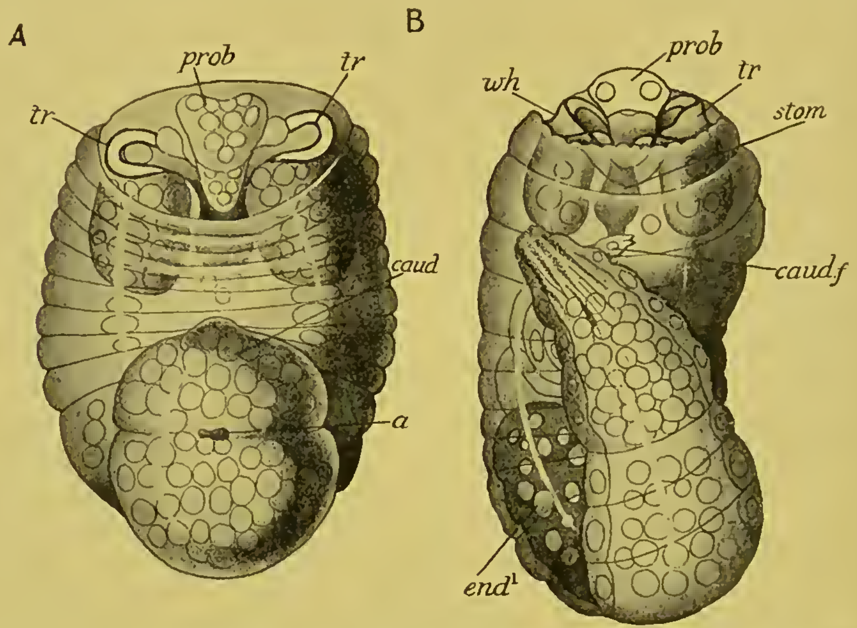

FIG. 337.—Ventral views of two embryos of *Callidina russcola* at a stage not long before hatching. (After Zelinka.)

A, younger stage: stage of invagination of rudiments of wheel-organs. B, older stage: stage of evagination of rudiments of the wheel-organs and of preponderant growth of the tail. *a*, anus; *caud*, tail; *caud.f*, caudal fork; *end*^{1}, endodermal stomach; *prob*, rudiment of proboscis; *stom*, secondary stomodaeum; *tr*, cuticular band marking place where cilia of the "wheels" will appear later.

The first traces of the **excretory organs** appear comparatively later as two streaks of dull glistening cells, situated at the sides of the pharynx. Subsequently they are developed into the coiled canal and adherent solenocytes, of which in the adult there are eight on each side. The **bladder** into which they open is derived from the endoderm contained in the foot region; this endoderm has been pushed forward by the development of those ectoderm cells which constitute the foot gland.

AFFINITIES OF THE ROTIFERA

Zelinka endeavours to show that the course of development which he describes proves, that *Callidina*, and therefore presumably all other Rotifera, show affinities to the Trochophore larva of Annelida and Mollusca; that, in a sense, they are what Huxley termed persistent Trochophore larvae. That there is a general resemblance between certain Rotifers such as *Callidina* when adult, and Trochophore larvae cannot be denied; but if this resemblance be an indication of real affinity, then we must regard the early development of Rotifera as profoundly modified; for there is no resemblance between the early development of this group and the early development of a typical Trochophore larva, with its specialized cleavage, characterized by the formation of four macromeres and three quartettes of ectodermic micromeres given off from near the animal pole of the egg. The small cells in the Rotiferan egg are given off, one by one, from the *vegetative pole*, and rotate round the posterior end of the egg on to the dorsal surface. The excretory organs of the Rotifera look like the archnephridia of the Trochophore larva, but their origin from the ectoderm has yet to be demonstrated. Unlike all other nephridia they do not open to the exterior, but discharge into an endodermic sac—the bladder, which we may compare to a **coelomic sac**, which still retains its opening into the gut. The early differentiation of the rudiments of the genital organs is a common feature in animals without larval development and with a very short life-cycle. On the whole, we may conclude that much more work remains to be done on the development of types of Rotifera with different forms of trochal disc, before the hypothesis of the affinity of the group with Trochophore larvae can be regarded as substantiated.

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CHAPTER XIV

CHAETOGNATHA

The little group of the Chaetognatha, or "Arrow-worms," consisting of *Sagitta, Spadella,* and *Krohnia,* is one of the most isolated in the animal kingdom, and its affinities are extremely difficult to determine. All we can say with certainty is that its members exhibit a very primitive type of adult structure and a still more primitive development.

SAGITTA

The Chaetognatha are with few exceptions pelagic animals and are of world-wide distribution. The development of the genus *Sagitta* has been worked at by Kowalevsky (1871), Bütschli (1873), and by Hertwig (1880), who described what could be made out from observations on the living embryos and from preserved specimens mounted whole. The subject has been taken up again by Doncaster (1902), who used the method of sections and who has given us a fairly complete account of the development of *Sagitta bipunctata.* It must be admitted, however, that some of Doncaster's figures are far from satisfactory, and it is greatly to be desired that a revision of the subject should be made.

Doncaster's method of procuring the eggs and embryos was, to secure a number of adults and keep them in a jar of clean sea-water, in which they lived very well for a day or two, and then to siphon off the bottom layer of the water and to look for the eggs in it. Doncaster preserved the eggs and larvae in a concentrated solution of corrosive sublimate to which 5 per cent of acetic acid was added. He used the method of double embedding in celloidin and paraffin, so frequently recommended in this volume and described in Chapter II.

Practically the whole development of *Sagitta* is completed within the egg-membrane, and when the embryo emerges and becomes a larva it differs from the adult chiefly in size and in the absence of developed genital organs. The whole development up to hatching only occupies about two days.

The egg, which is ·2 mm. in diameter, segments perfectly regularly into blastomeres of approximately equal size. A blastocoele appears at the 8-cell stage, and the result of segmentation is the production of a spherical hollow **blastula** which, it may be remarked incidentally, is not unlike the blastula of *Amphioxus*, although at once distinguishable from it by its greater size. One side of this blastula flattens and on the flat side an invagination takes place, and the blastula is thus converted into a **gastrula**.

The invagination, which is the rudiment of the archenteron, increases in depth till it practically fills up the entire blastocoele and the endoderm is pressed close against the ectoderm. The whole gastrula then elongates, the blastopore narrows, and the embryo assumes a fusiform shape, being more or less pointed at the anterior and posterior ends and broadest in the middle.

At the same time the mother cells of the **genital organs** make their appearance as a pair of large cells which project into the cavity of the archenteron; they occur in a position about one-fourth the length of the embryo from the anterior end. They are extruded from the wall of the archenteron and are therefore endodermal in origin (Fig. 338). They touch each other and are, of course, situated symmetrically with regard to the median sagittal plane of the embryo, but, according to Doncaster, they are nearer either the dorsal or the ventral surface of the embryo; he could not decide which, for he was unable to find any definite landmark to discriminate between dorsal and ventral surfaces. The very early appearance, as peculiar cells, of the rudiments of the genital organs in many groups, such as those treated in this and in the preceding and following chapters, has led to interesting speculations as to whether a reproductive substance is not separated from a somatic substance at the very beginning of development. This problem has been attacked, in the case of the development of *Sagitta*, by several authors, of whom Buchner has

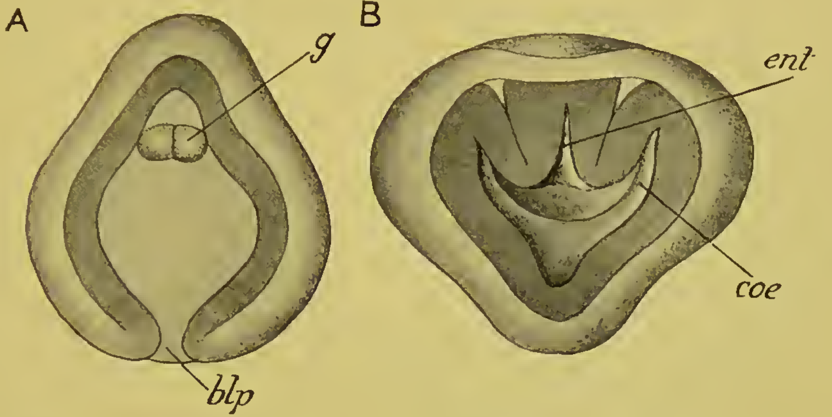

Fig. 338.—Optical sections of early embryos of *Sagitta bipunctata*. (After Doncaster.)

A, gastrula showing the two mother cells of the genital organs. B, embryo in which the archenteron is being divided by lateral folds into coelomic sacs at the sides and enteron in the middle. *blp*, blastopore; *coe*, coelomic sac; *ent*, enteron; *g*, mother cell of the genital organs.

given the most recent and satisfactory account (1910). According to him, during the ripening of the egg, one of the primitive germ cells becomes embedded in its cytoplasm and degenerates, leaving, as remains, a deeply staining oval body (*nut*, Fig. 339). When the egg undergoes segmentation this body is found in one of the first two blastomeres. In subsequent divisions it is not divided, but always passes into one blastomere. When, however, the blastula stage is passed and gastrulation has been accomplished, it is divided between two cells, and *these cells are the mother cells of the genital organs.* It thus appears that in *Sagitta*, as in the Crustacean *Polyphemus* (see p. 193), the cell destined to produce the genital organs is distinguished, not by peculiarities of its nuclear substance, but by the possession of an extra store of nourishment.

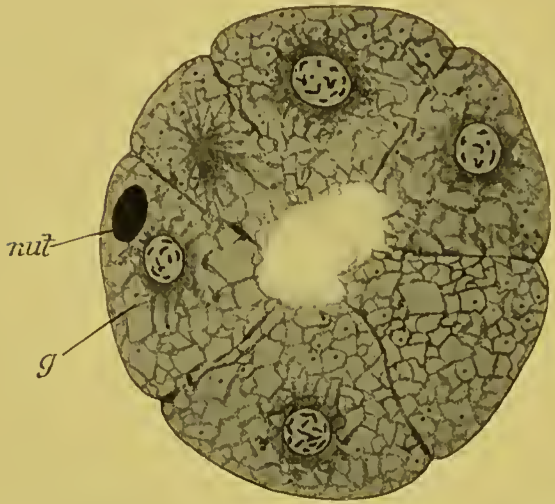

FIG. 339.—Cross section of the blastula of *Sagitta bipunctata* (?) in the 16-cell stage, showing the determination of the mother cell of the genital organs. (After Buchner.)

g, mother cell of the genital organs; *nut*, remains of nutritive cell embedded in the mother cell of the genital organs.

The next change which occurs in the development is the formation of the **coelom**. This takes place by the development of a pair of inwardly directed folds in the archenteric wall, which start from its anterior border, one on each side of the middle line, and grow backwards as a double wall. In this way the archenteron becomes divided into a median chamber, the digestive tract or gut, and two lateral chambers, one on each side, the coelomic sacs. At first all three chambers open into a common undivided chamber behind.

At the same time the shape of the embryo changes, because as these folds develop they use up a considerable portion of the front part of the archenteric wall, which had projected forward in a conical process. As a consequence, the anterior pointed end of the embryo becomes changed into a broad, very slightly convex surface, whilst the posterior end remains pointed.

The mother cells of the genital organs are in contact with the archenteric wall almost at the point where the folds originate, and as these grow backward they carry the mother cells of the genital organs before them, so that these primitive germ cells are carried back into the hinder half of the embryo. There they pass into the coelomic division of the archenteron on each side, round the bend of the fold, and each then divides into an anterior and a posterior half (g_1, g_2, Fig. 340).

Whilst this has been occurring the whole embryo grows considerably in length, so that the anterior convex end bears but a small proportion to the total length. The central division of the archenteron becomes closed off from the two lateral divisions behind, by the approximation and union of the inner walls of its folds, and in this way the **gut** is separated from the coelomic sacs.

The front portions of the coelomic sacs, lying at the sides of the stomodaeum, become separated from the rest and form a pair of round thick-walled **head-cavities**. The cavities are soon obliterated, but their walls give rise to the muscles which move the curved setae situated near the mouth, which give the name to the phylum.

The masses of mesoderm which represent the head-cavities touch each other dorsally, but ventrally are separated by the stomodaeum, so that they resemble a horse shoe in shape. Traced backwards these masses of head-mesoderm are seen to overlap the front ends of the hinder portions of the coelomic sacs which are nearer the middle line.

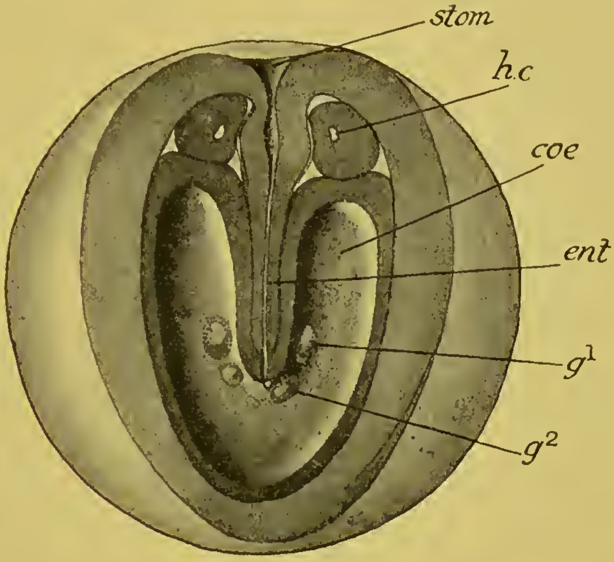

FIG. 340.—Optical section of older embryo of *Sagitta bipunctata*, showing the formation of the "head-cavities" and of the stomodaeum. (After Doncaster.)

coe, coelomic sac; *ent*, enteron; g^1, mother cell of ovary; g^2, mother cell of the testis; *h.c*, head-cavity; *stom*, stomodaeum.

The blastopore closes completely; its last trace is on the ventral surface, far back but not quite at the posterior end, and at the same time an ectodermic invagination makes its appearance at the anterior end of the embryo; this is the **stomodaeum**, which grows backwards and breaks through into the gut.

The coelomic sacs continue for a short time to open into a common sac behind, which occupies about one-third the length of the whole embryo, but soon this single sac becomes divided into right and left halves by the backward growth of a septum. This septum is formed by the coalescence of the right and left archenteric folds, and it might almost be regarded as a solid portion of the gut.

The adult *Sagitta* exhibits a differentiation of the body into head, trunk, and tail. The head swelling is *initiated* in the embryo by a great anterior dorsal ectodermic proliferation, which later gives rise to the **brain** or **supra-oesophageal ganglion** (*s.o.g*, Fig. 341). It is *increased* by the formation of a lateral swelling on each side, forming a ridge. These two ridges later unite to form the **cephalic hood**. Finally, owing to the fact that, as the embryo increases in length, the stomodaeum retains its cavity whilst the rest of the gut becomes compressed to form a vertical solid band, and the cavities of the coelomic sacs also temporarily disappear, the breadth of the head region as contrasted with that of the trunk is emphasized, and the head swelling stands out distinctly. This suppression of the cavities seems to be largely due to the circumstance that, whilst the embryo is growing in length and becoming curved, it is still confined within the egg-shell.

The **ventral ganglion** makes its appearance as a thickening of the ectoderm, stretching from immediately behind the head to nearly the region where the anus is formed, and taking up two-thirds of the length of the embryo (*v.g*, Fig. 341). The ectoderm cells become

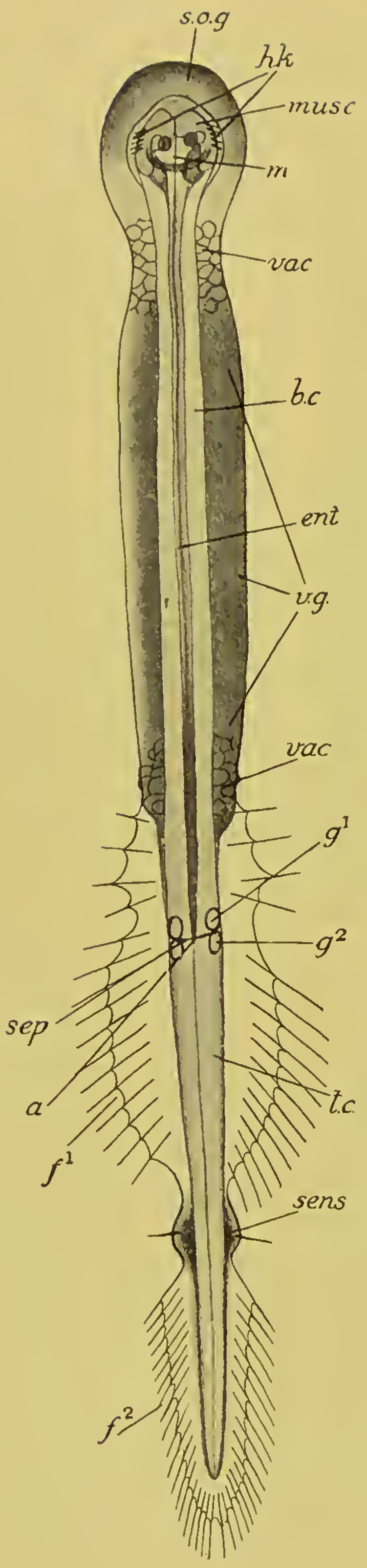

FIG. 341.—Ventral view. Larva of *Sagitta enflata* on the fourth day after hatching, in order to show the origin of the nervous system and the separation of the trunk coelom from the tail coelom. (After Doncaster.)

a, position of the anus; *b.c*, body coelom; *ent*, alimentary canal; f^1, f^2, anterior and posterior fins; g^1, mother cell of ovary; g^2, mother cell of the testes; *hk*, masticatory hooks; *m*, mouth; *musc*, masticatory muscles developed from the head-cavities; *sens*, sense-organ (tactile?); *sep*, transverse septum dividing body coelom from tail coelom; *s.o.g*, supra-oesophageal ganglion; *t.c*, trunk coelom; *vac*, vacuolated cells (floating tissue); *v.g*, ventral ganglion.

columnar in this region and their nuclei retreat to their bases, where they undergo rapid division and give rise to small rounded ganglion cells. This proliferation does not take place evenly all over the ventral surface but along two parallel lines, and we are involuntarily reminded of the double nature of the Annelid ventral nerve cord. Sparsely scattered over the body are groups of sensory hairs, arranged like fans, transversely to the long axis of the body. These are more numerous on the head. At their bases groups of sensory nuclei can be seen.

At this stage the young *Sagitta* is hatched. If the foregoing description has been followed, it will be seen that it is a solid worm very unlike the adult, and therefore merits the name of larva. It is about 1 mm. long, and it swims near the surface of the water, propelling itself by lateral jerks of the tail fin, which is already developed. At the bases of the mesoderm cells in the trunk and tail the fine refractive muscular fibrils can already be made out. They all run longitudinally.

After swimming for about two days the larva undergoes a comparatively rapid change which makes it much more nearly like the adult in structure. Cavities suddenly reappear both in the mesoderm of the trunk and in that of the head, and so the larva becomes inflated and more transparent.

The cephalic hood becomes a prominent fold, and under it a pair of **hooks** is to be seen on each side. These hooks gradually increase in number by the addition of new ones in front, till the full number characteristic of the species is attained. At each side of the mouth a rounded ectodermic thickening appears, which is the so-called **lateral ganglion.** The eyes also appear as minute black specks. The cavities in the head disappear again, and the head mesodermal cells become arranged in definite bundles, forming muscles for moving the hooks.

As the larva grows in length the ventral ganglion becomes relatively shorter and the ectoderm cells in front and behind it become vacuolated so as to form a kind of floating tissue (*vac*, Fig. 341). Such vacuolated cells are found all over the trunk throughout life in *Spadella draco*, but of course they disappear later in life in *Sagitta*.

In the tail region the tail fin becomes large and prominent and extends a good distance forward. It is separated by a gap from two lateral fins which have appeared, one on each side. In the gap on each side is situated a large **tactile organ**, borne on an ectodermal prominence (*sens*, Fig. 341).

On the fourth day the genital cells, which up till now have been disposed in two pairs, an anterior and a posterior pair on each side, begin to migrate across the body-cavity from its splanchnic to its somatic wall. As this takes place a **lateral septum** is formed, cutting the coelom on each side into trunk and tail portions, and separating the anterior genital cells on each side, which are the rudiments of the **ovaries**, from the posterior ones, which are the rudiments of the **testes.** This septum is probably formed as an outwardly projecting fold of the splanchnic mesoderm, possibly it may be a modification of portions of the thin envelopes of the genital cells, which envelopes are themselves thin layers of cells derived from the splanchnic layer of the mesoderm.

According to Doncaster, the hood which surrounds the head is formed by a splitting off of an outer layer of cells from the ectodermic thickening on each side, and the head coelom persists for a considerable time and it is its outer wall which is metamorphosed into the masticatory muscles.

The alimentary canal reacquires its cavity about the eighth day, and on this day its posterior end bends ventrally and comes into contact with the ectoderm, where the anus is formed. On the head at the same time there appears a double curved line of closely packed nuclei, in the form of a horse-shoe open backwards, and this constitutes the **olfactory organ.**

Doncaster was unable to keep the young *Sagitta* alive for a longer period than fifteen days, and up till this period no further changes had taken place in the genital rudiments. The further development of these organs was determined by fishing young individuals from the Plankton.

By a comparison of these with the latest stages reared from the egg it is seen that each of the four primary genital cells divides so as to form a little mass of cells. The masses of cells representing the ovaries grow forwards, those representing the testes grow backwards. Incomplete longitudinal septa appear in the tail coelom on each side, and bunches of cells drop off from the testes and float in the tail coelomic cavity, where they undergo all the changes which lead to their transformation into mature spermatozoa.

The **vasa deferentia** appear as ectodermic thickenings in the space between the lateral fin and the tail fin on each side. This thickening splits into two layers with a cavity between them. In front this cavity is exceedingly narrow, and here it eventually acquires an opening into the coelom. Behind it acquires an opening to the exterior, and this opening is formed at a spot in the course of a longitudinal groove which appears on its outer wall.

The ovary becomes differentiated into two well-defined layers, an inner and outer, between which is a loose ill-defined mass of cells. The outer layer resembles an epithelium and consists of small cubical cells abutting against the outer wall of the trunk coelom. The inner layer consists of tall columnar cells which are the mother cells of the ova.

As the ova enlarge and mature they become pressed out of this layer of epithelium, and are found projecting from the surface of the ovary towards the body-cavity, though covered with a structureless membrane. The loose cells which intervene, especially at the base of the ovary between the germinal epithelium and the ectoderm, eventually develop a cavity and form the **oviduct.** This grows backwards and at maturity reaches and fuses with the ectoderm at a point where the female opening is formed. Buchner (1910) has found that the large ova pass into the narrow oviduct through narrow, slit-like openings which are only visible at the moment of passage. From the description just given it follows that the male ducts are of ectodermal origin, but that the origin of the female ducts is doubtful, for the precise origin of the loose cells which give rise to them has not been ascertained.

AFFINITIES OF THE CHAETOGNATHA

As mentioned in the beginning, the affinities of the Chaetognatha are very obscure. The early development so far, the segmentation of the egg, the formation of the archenteron by invagination, and the formation of the coelom as lobes of the archenteron, is of a very primitive type and has been compared to that of Brachiopoda and Echinodermata. What justice there may be in the latter comparison will be considered when the development of Echinodermata is described.

With regard to Brachiopoda, Conklin lays stress on the difference between a single anterior septum dividing gut from coelom in Brachiopoda and the two lateral folds in Chaetognatha, but this difference is annulled if, as Kowalevsky describes, the coelom in the Brachiopod *Argiope* is separated from the gut by two lateral septa. But the posterior position of the last traces of the blastopore, and the early appearance of the stomodaeum, are two great differences in development which separate Chaetognatha from Brachiopoda.

Another great difference is the total absence of cilia in the embryo of *Sagitta*. This is probably partly correlated with the fact that the development of *Sagitta*, in spite of its primitive features, must be extremely shortened, for the larva, though it differs from the adult in its functionless alimentary canal and solid mesoderm, nevertheless has the same mode of life as the adult. On the other hand, in Brachiopoda, as indeed in nearly all the groups of the animal kingdom which we have as yet studied, the larva has quite a different mode of life and different locomotor organs from the adult. The very early differentiation of the genital cells is another sign of shortened development.

Hertwig compared the Chaetognatha to an Annelid with three segments, and a comparison has also been suggested between the three pairs of body-cavities in Chaetognatha and the three segments of the Brachiopod larva. But with whatever justice such a comparison can be applied to the head and trunk segments, it is doubtful, as Doncaster wisely remarks, how far this explanation can be applied to the separation of trunk and tail segments; for this latter separation occurs in Chaetognatha long after the separation of the head-cavities, and it appears to be a device to separate male and female reproductive organs.

Günther (1907) has written a long paper to try to prove that *Sagitta* is related to the stem of the Mollusca. As the parent stems of Mollusca and of Annelida are identical, this conclusion is not so different from that of Hertwig as might at first sight appear to be the case. But Günther's conclusions are based entirely on interpretations of adult anatomy and ignore the question of development, in which *Sagitta* differs absolutely from any Mollusc so far studied. The facts that *Sagitta* is essentially a chitinous animal which nowhere develops cilia, and that its locomotor organs are both developments of this cuticle, constitute a deep gulf between it and any of the soft-skinned Mollusca.

It has been suggested that the Nematoda have affinities with the Chaetognatha. The arguments for and against this will be considered in the next chapter, when the development of Nematoda will be described. But even if we should finally decide that such an affinity exists, it would throw no light on the origin of Chaetognatha, because in that case the Nematoda would have to be regarded as much modified and degenerate Chaetognatha which had taken to burrowing and parasitic habits.

The conclusion to which we are led, the arguments for which will be developed later, is, that in immensely remote times there existed a stock of free-swimming animals in which the coelom still was in open communication with the gut. This hypothetical stock may be called the Protocoelomata, and from it all the groups of coelomate animals would be descended. If this be correct, the Chaetognatha would represent a small, comparatively unmodified offshoot from that stock, a group which has been able to hold its own until the present day.

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CHAPTER XV

NEMATODA

The Nematoda, often called Thread-worms or Round-worms, are a group in which there is wide diversity of habitat and life-history, but most remarkable uniformity in adult structure. The vast majority are parasitic during some period of their existence; it is indeed by no means certain that even among the so-called free-living Nematodes many have not a parasitic stage in their life-history, because this history is very far from being completely known. Though they infest the most diverse animals and plants, the structure of the young worm, before the genital organs are developed, is remarkably constant throughout the group; and so is the embryonic development so far as it is known. Owing to the fact that the eggs and embryos are very minute, the method of sections has been hardly at all applied to the study of the group; most observations have been made on the living embryo seen through the semi-transparent egg-shell, whilst others have been made on embryos fixed, stained, and mounted whole.

In spite, therefore, of numerous investigations, much remains obscure in the embryology of these worms. Indeed it must be said that most of these investigations have had for their object the solution of general problems, such as the relative importance of cytoplasm and nucleus in heredity, the relationship of genital and somatic cells, the nature of the polar bodies, etc., rather than the elucidation of the special course which development takes in this group, and the light which it throws on the affinities of the group. These words are not said to disparage the painfully laborious investigations which have already been made, but to indicate that there is still a promising field left for future investigators.

ASCARIS MEGALOCEPHALA

The form whose study has so far yielded the most satisfactory results is *Ascaris megalocephala*, a large Nematode, about 9 or 10 inches long, which inhabits the intestine of the horse and causes no noticeable ill effects. It is readily obtained in great numbers at the abattoirs.

The eggs are contained in the two uteri. They do not commence to develop until they have been laid, but once they have commenced to develop they are wonderfully tenacious of life. If they are spread on slides and attached to the slide with albumen fixative, and the slides are then put into a moist chamber or even into weak formalin, their vitality is quite unimpaired and they pursue an orderly course of development. This can be suspended by exposing them to cold, and when the temperature rises the development is resumed at the point at which it left off. This circumstance makes the development of *Ascaris* an extremely convenient subject to work at for a professor burdened with professional duties; when he has leisure the development is allowed to proceed, when he is busy with other duties the development is suspended by exposing the eggs to cold.

A word or two on the adult anatomy might be in place here. It is characteristic of most Nematoda to have two female organs, which open by a pore situated on the mid-ventral line about one-third the length of the body from the front end. Each of these organs consists of a long tube. The distal end of this tube is practically solid and contains large nuclei embedded in a mass of cytoplasm, in which cell divisions are not obvious. As one proceeds farther down the tube a cavity appears, and the cytoplasmic territories of the various nuclei become delimited from each other so that we can speak of a layer of cells surrounding a cavity.

These two sections of the tube are known as the **ovary**. Below this the ova become detached from the lining of the tube and lie in its cavity. This portion is termed the **oviduct**. Here the maturation divisions take place and the male cells meet and unite with the female cells. Below this the tube widens and the eggs become surrounded by their horny egg-shells, in which they complete most of their development. This section is termed the **uterus**. Finally there is an extremely short terminal piece common to the two tubes and known as the **vagina**.

The sexes are separate in *Ascaris megalocephala* as in the great majority of Nematoda. The male organ consists of a single tube which, in a manner similar to the topography employed in the case of the female organs, is mapped out into a **testis**, without cavity and with undivided cytoplasm and numerous nuclei, a hollow **vas deferens**, in which the male cells become detached and mature, and a terminal **vesicula seminalis** in which the mature male cells are stored up. This tube opens near the posterior end into an ectodermal pit or atrium, the **cloaca**, into which the posterior end of the alimentary canal also opens.

Two horny spicules, inserted in ectodermal pockets of the cloaca at the sides of the male genital opening and capable of being extruded by special muscles, are used to distend the genital opening of the female and effect fertilization. These are known as **copulatory spicules**.

The male cells of Nematoda are not spermatozoa but small amoeboid cells with large nuclei. By carefully opening mature specimens of *Ascaris megalocephala* and unravelling the genital tubes so gently as not to break or injure them, by then carefully dividing these tubes into short lengths which are numbered so as to show the position of each portion in the tube from which it was taken, and by preserving these portions in separate bottles, material may be obtained for an exhaustive study of the maturation of male and female cells, and it was in this way that Hertwig in 1890 obtained evidence as to the nature of the polar bodies.

It is a most remarkable fact that there are two races of *Ascaris megalocephala,* in one of which there are four chromosomes in the oogonia and spermatogonia, and two chromosomes in the ripe eggs and male cells; and in the other of which there are two chromosomes in the oogonia and spermatogonia, and one chromosome only in the nucleus of the ripe eggs and male cells. The first variety is called *bivalens,* the second *monovalens.* The genital cells at any given cross section of the genital tube are all in the same stage of development.

The most exhaustive studies of the development of the fertilized egg have been made by Zur Strassen (1896) and by Boveri (1899), who observed the development up to about the 200-cell stage, also by the former's pupil Müller (1903), who endeavoured to carry on the analysis of the development up till the time when the young worm was hatched.

Zur Strassen's method of making whole mounts of the eggs and embryos was as follows. The eggs were fixed by being placed for twenty-four hours in a mixture of four parts of 95 per cent alcohol and one part glacial acetic acid; they were then stained for twenty-four hours in Grenacher's hydrochloric acid carmine; the excess of stain was removed by immersion for forty-five minutes in 95 per cent alcohol to which 1 per cent of hydrochloric acid had been added. They were then washed in 95 per cent alcohol. Some glycerine was added to this, and by allowing the alcohol to evaporate gradually the eggs were got into a solution of pure glycerine. The eggs were examined in this medium, and by gently pressing on the coverslip they could be rolled into various positions and examined from all sides. Boveri recommends similar methods, but he also employed picro-acetic acid, made by adding to a concentrated solution of picric acid in water, two volumes of water and 1 per cent of glacial acetic acid.

The segmentation of the egg of *Ascaris megalocephala* is by far the most specialized development that has ever been described. Many workers besides Zur Strassen have examined it, and a nomenclature has been gradually agreed on, which will be employed here. This nomenclature was not fully developed when Zur Strassen wrote his long descriptive paper, but it is easy to translate the nomenclature which he there employs into the more modern nomenclature.

The egg divides into two blastomeres, an upper and a lower. The upper, which is designated AB, is slightly larger and freer from yolk granules than the lower, which is designated P_1. The nucleus of AB undergoes an extraordinary change known as the "**diminution of the chromatin**" (Fig. 342, B). At the moment that it separates from its sister nucleus in P_1, it contains, like the fertilized egg, four chromosomes (or two in the variety *monovalens*). But as it passes into the resting stage, the greater portion of each of these chromosomes is cast out as amorphous masses of chromatin, which for a time can be recognized in the cytoplasm but which are gradually absorbed. The remainder of each chromosome breaks up into a number of minute granules.

When AB next divides each of these granules acts like a minute chromosome, and hence the spindle that is formed exhibits a totally different appearance from the spindle that is formed in P_1, which shows, of course, the ordinary four (or two) chromosomes.

At the next cleavage AB divides into an anterior cell A and a posterior cell B, whilst P_1 divides into an upper cell denominated EMST and a lower cell P_2. The nucleus in EMST undergoes a reduction precisely similar to that undergone by the nucleus in AB. The embryo is now shaped like a T. The two lower cells bend upwards first in a plane at right angles to the plane of the two upper cells; they then, however, swing round into the same plane, and so the T becomes a rhomb, *and the plane of this rhomb is the future median plane of the embryo.*

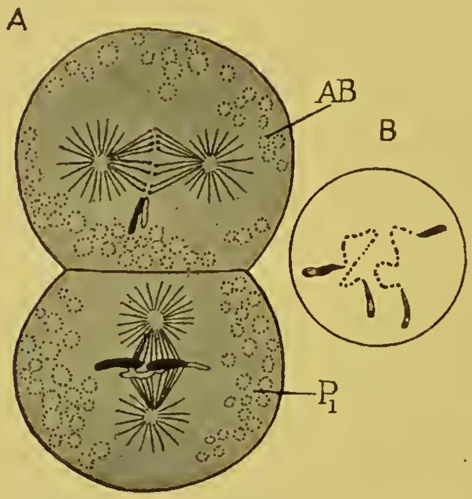

FIG. 342.—The 2-cell stage of the egg of *Ascaris megalocephala* (*monovalens*) when the spindles for the 4-cell stage are being formed, showing diminution of the chromatin. (After Boveri.)

A, the 2-cell stage seen from the side. B, two chromosomes from the upper cell enlarged to show the process of diminution of the chromatin.

At the next cleavage A divides into a right cell named *a* and a left cell named *α*. B divides similarly into a right cell named *b* and a left cell named *β*. EMST divides into an anterior cell named MST and a posterior one named E. P_2 divides into an anterior cell called P_3 and a posterior one called C. The nucleus in C undergoes reduction so that only P_3 retains large chromosomes. The four cells MST, E, P_3, and C follow each other in a curve, convex below, which lies in the median plane of the embryo. They and their descendants are known as the **ventral cell family**, whilst *a*, *b*, *α*, and *β*, and their descendants, are known as the **dorsal cell family**.

The dorsal cell family divides much more rapidly than the ventral cell family, but before any further divisions take place certain characteristic rearrangements of the members of the dorsal family occur. On the right side a glides upwards and b downwards, whilst on the left side α glides downwards and β upwards (Fig. 343, D).

At the next cleavage all four cells divide and the two daughters of each are denominated 1 and 2, the more dorsal sister being indicated

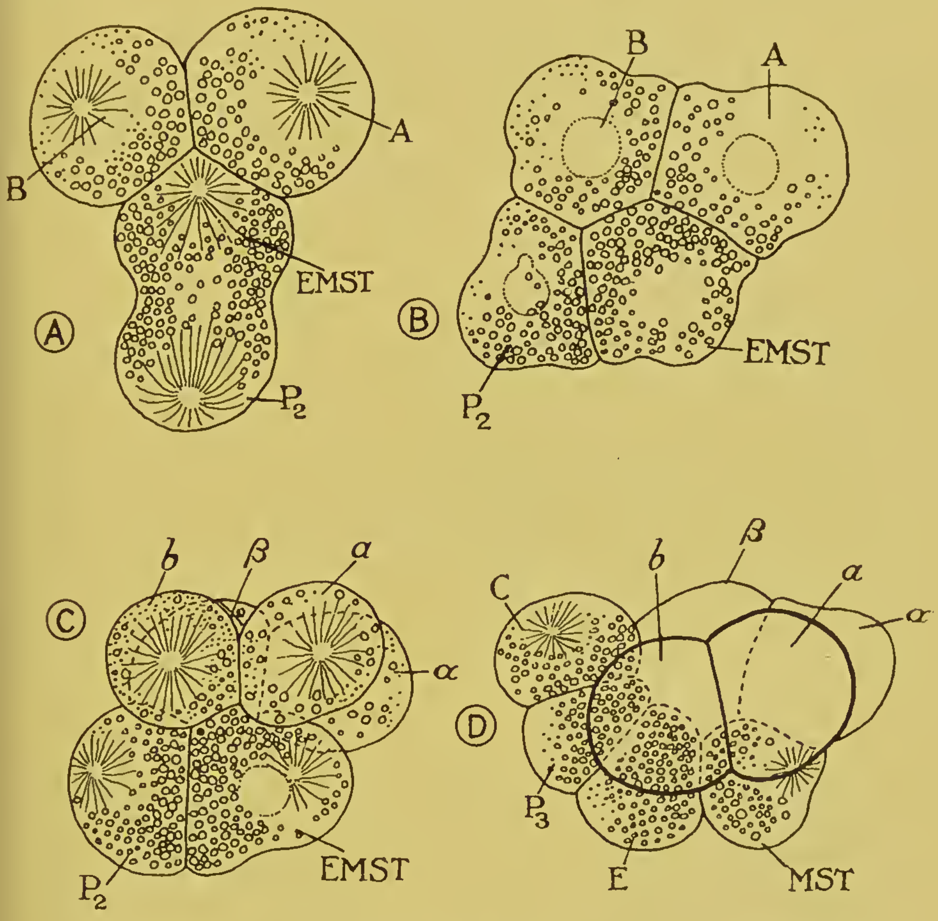

FIG. 343.—Early stages in the segmentation of the egg of *Ascaris megalocephala*. (After Boveri.)

A, 4-cell stage in the T-condition seen from the right side. B, 4-cell stage in the rhomb condition seen from the right side. C, 6-cell stage seen from the right side. D, 8-cell stage seen from the right side.

by the suffix (1). As a result of the gliding that has taken place in the previous stage, the four cells of the upper cell family on the right side form a T-piece with a horizontal beam and a vertical cross-piece. The beam consists of b_1 in front and b_2 behind, the cross-piece of a_1 above and a_2 below. The four cells on the left side form a rhomb α_1 and α_2, lying rather in front of, but beneath, β_1 and β_2. The four cells of the ventral cell family form a horse-shoe with approximated ends. Then a fresh rearrangement takes place, α_1 passes to the middle line between a_1 and a_2; a_2 is below and in front, whilst a_1 is above and behind; the effect of this is to force apart the limbs of the horse-shoe formed by the ventral cell family and to force the terminal cell C backwards and downwards (Fig. 344). A new T-piece is then formed, which persists throughout the whole development. The cross-piece of this T is horizontal and is made by the two cells a_2 and α_2, the beam is constituted by the two cells a_1 and α_1 (Fig. 345).

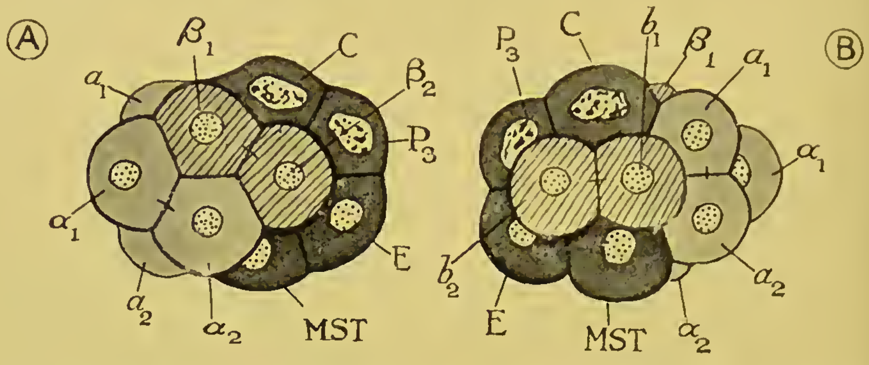

FIG. 344.—The 12-cell stage of the segmentation of the egg of *Ascaris megalocephala*, seen from the left and the right sides respectively in order to show the rearrangement of the daughters of the cell AB. (After Zur Strassen.)

A, from the left side. B, from the right side. The daughters of A have the lightest tone. Those of B have the same tone crossed with oblique lines. The ventral cell family have the deepest tone.

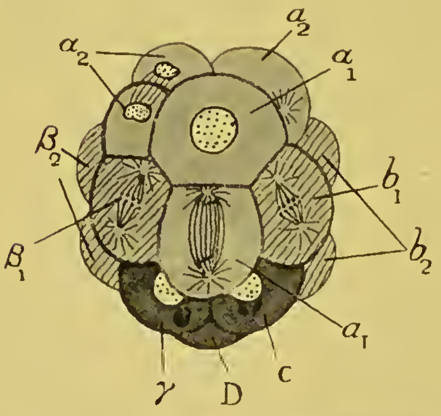

FIG. 345.—Dorsal view of the segmenting egg of *Ascaris megalocephala* in the 20-cell stage. (After Zur Strassen.)

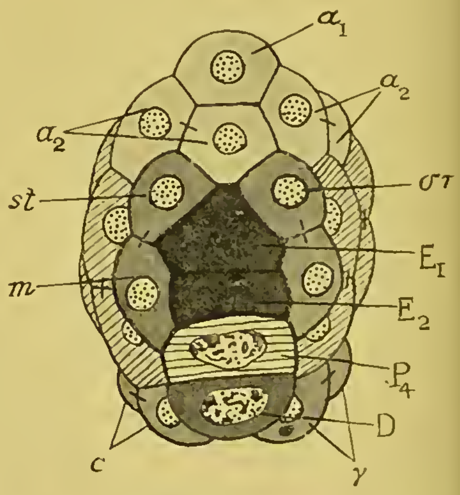

FIG. 346.—Ventral view of the segmenting egg of *Ascaris megalocephala* in the 24-cell stage. (After Zur Strassen.)

In this stage the embryo is an egg-shaped blastula.

Then the cells of the ventral cell family divide. MST divides into a right cell denominated *mst*, and a left one denominated *μστ*. E divides into E_1 in front and E_2 behind, P_3 divides into P_4 in front and D behind, and C divides into a right cell *c* and a left cell γ.

The nucleus of D undergoes reduction of chromatin, so that finally only the cell P_4 is left in which the chromosomes retain their original condition. This cell in the subsequent development divides only once until the larval period is attained, and it is the parent cell of the **genital organs** and gives rise to nothing else.

It follows, therefore, that the tissues of the Nematode are constructed of cells which have lost a large part of the chromatin which they had inherited from the egg cell, and which are, therefore, as compared with the germ mother cell, degenerate cells.

Zur Strassen has followed in detail the subsequent divisions of the dorsal cell family, adopting for his purpose a most complicated nomenclature, but nothing is gained by following him because there is some evidence to show that these cells are equivalent to each other, and that if, through abnormal circumstances, their number is increased, it does not affect the normal development of the resulting embryo. The daughters of A, as we have seen, form a median T-piece, and the two sides of the beam of this are occupied by the daughters of *b* and *β* respectively.

Zur Strassen's object was to discover if the mutual displacements of the cells of this family could be accounted for by the laws of surface-tension, or whether there was evidence of cytotaxis, *i.e.* mutual attractions and repulsions of cells based on their chemical qualities. He found that surface-tension did undoubtedly cause rearrangement; it caused the cells to arrange themselves according to Plateau's principle, *i.e.* in such a way that the total area of their boundary surfaces was reduced to a minimum; but he also found that it was not the sole principle at work, because in some cases cells moved in such a way as to directly contravene that principle, and hence there was evidence that cytotaxis was at work (Fig. 347).

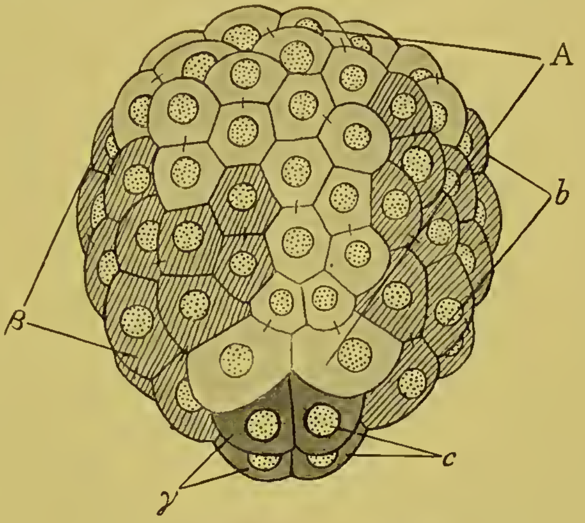

Fig. 347.—Dorsal view of the segmenting egg of *Ascaris megalocephala* in the 102-cell stage. (After Zur Strassen.)

The daughters of A forming the permanent cross are light-coloured. Those of *b* and *β* are cross-hatched.

Returning to the last stage minutely described, when both dorsal family and ventral family consisted of eight cells, *i.e.* the 16-cell stage, we may note that at this stage the egg forms an elliptical **blastula** with a well-marked **blastocoele.** The first trace of the invagination which will change the blastula into a **gastrula** is seen in the cell E, which is the mother cell of the endoderm. Before this cell divides into E_1 and E_2 the nucleus shows a tendency to migrate inwards and upwards, but when it divides the daughter nuclei lie at the ordinary level.

At the next division of the cells of the ventral family, *mst* divides into an anterior cell *st*, which is one of the cells which form the **stomodaeum**, and a posterior and lateral cell which is one of the mother cells of the **anterior mesoderm.** $\mu\sigma\tau$ similarly divides into a stomodaeal cell $\sigma\tau$ and a mesoderm mother cell μ (Fig. 346). E_1 and E_2 divide into right and left daughters which (following Plateau's principle) assume a rhombic arrangement. The two tail cells c and γ each divide into an anterior and a posterior cell, and the cell D also divides into right and left daughters. The nuclei of all four endoderm cells take up positions near the inner margin of their respective cells, the cytoplasm follows suit, and so the process of **gastrulation** is initiated (Fig. 348).

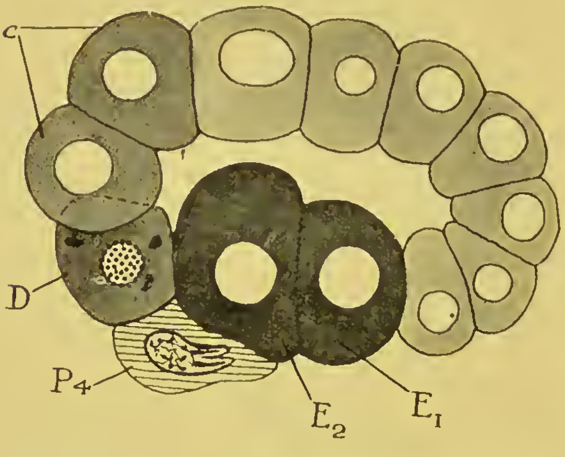

FIG. 348.—Optical median sagittal section through the blastula of *Ascaris megalocephala* at the time that the process of gastrulation is commencing. (After Boveri.)

The cells P_4 and D are beginning to extend beneath E_2.

In the next stage P_4 divides into an anterior G_1 and a posterior G_2. These two cells remain quiescent and undergo no further division until after the larva is hatched and the development of the genital organs is begun. Boveri, however, maintains that sometimes, at any rate, the hinder of these cells divides into right and left daughters which are ectodermal, and that the two mother germ cells arise by a second division of the front cell. The anterior pair of tail cells divide each into two daughters lying side by side, so that a transverse row of four is formed, but the posterior tail cells divide each into two, one behind the other. The stomodaeal cells each divide into anterior and posterior daughter cells, and slightly later the anterior mesoderm cells follow suit. These mesoderm cells now pass into the blastocoele and lie between ectoderm and endoderm on each side (Fig. 351).

In the next cleavage both stomodaeal cells and mesoderm cells are raised to four on each side. In this way two short **mesodermal bands** are formed, each consisting of four cells, but each band contracts so as to form a rhomb. The endoderm cells increase to eight. The daughters of D (d and δ) divide each into two cells, lying side by side, so as to form a transverse line of four cells parallel to the row formed by the front tail cells.

Next, the second pair of stomodaeal cells come into contact with each other, the first pair are in contact from their first formation, and this group of four sinks inwards. Of the posterior group of the tail cells each divides into anterior and posterior daughters, and in this way a double row of eight is formed (*i.e.* four pairs, one behind the other). Each transverse row of four, *i.e.* the row formed from the front tail cells and the one formed from D, folds up into a U-shape. The hinder U merely continues the line of the tail cells, but the front one sinks inwards and forms, as Müller has asserted,

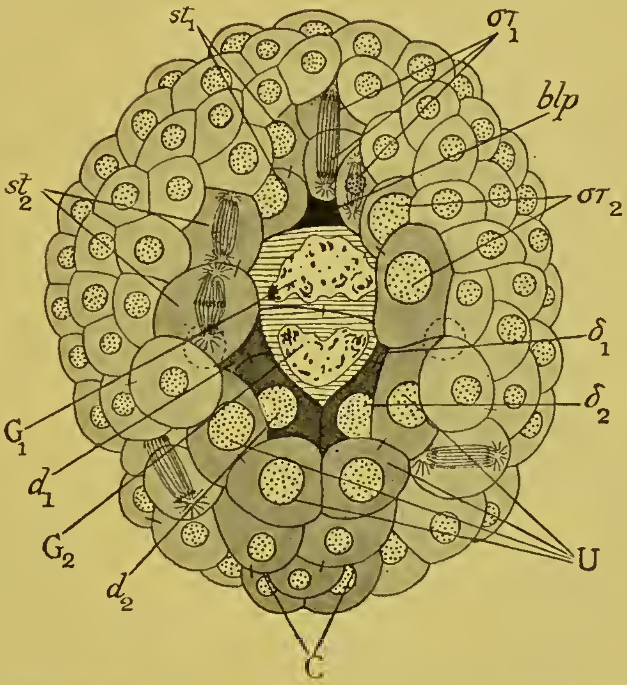

Fig. 349.—Segmenting egg of *Ascaris megalocephala* in the 102-cell stage seen from beneath. (After Müller.)

All the ectoderm cells which are descendants of AB have a light tone—the descendants of A and those of B are not distinguished from one another. *blp*, blastopore. U, the front row of tail cells descendants of C, which bend up so as to form a U. A similar U in front of this one is formed by the descendants of D (d_1, d_2, δ_1, δ_2) and gives rise to mesoderm.

a **posterior mesoderm** rudiment. (Zur Strassen had imagined that it gave rise to the proctodaeum, but this is an error.) Müller further asserts that a third mesoderm rudiment is formed, at a later stage, from some of the tail cells (Fig. 349).

Zur Strassen maintains that the tail cells, the descendants of C, are distinguishable from the descendants of AB, which he denominates the primary ectoderm, by their clearer protoplasm and flatter shape; and he maintains that, as development proceeds, the tail cells progressively displace the primary ectoderm cells till these last form only a small cap at the anterior end of the embryonic worm. Müller, however, has not been able to confirm this. The blastopore closes by the gradual approximation of its sides, and it is for a brief period long and slit-like (Figs. 350, 351).

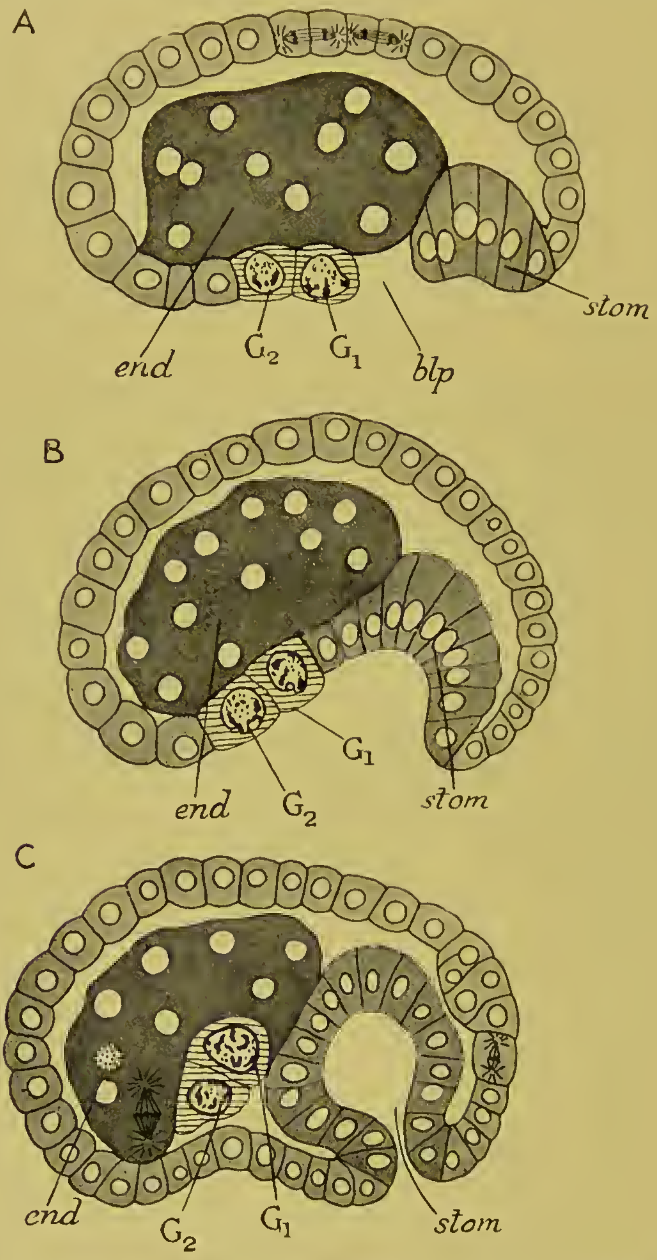

Fig. 350.—Median sagittal sections through three embryos of *Ascaris megalocephala*, in order to show the invagination of the genital cells and of the stomodaeal rudiment. (After Boveri.)

A, embryo at the close of gastrulation; a small part of the endoderm is still uncovered. B, embryo in which gastrulation is complete; the stomodaeal cells begin to be invaginated. C, embryo in which the stomodaeal cells form a tube, and in which the mother cells of the genital organs are covered in by ectoderm. *blp*, blastopore; *end*, endoderm; *stom*, stomodaeum.

As the tail cells multiply and the embryo elongates, a remarkable rearrangement takes place in some of the ectoderm cells covering the dorsal surface. These at first form two rows interdigitating with each other, but they quickly rearrange themselves so as to form a single row of band-like cells. Müller has observed two large clear cells lying on the ventral surface in the anterior part of the animal. These he surmises to be the mother cells of the **excretory tubes.** Although these tubes extend in the adult through nearly the entire length of the animal, they are each composed of a single hollowed-out cell.

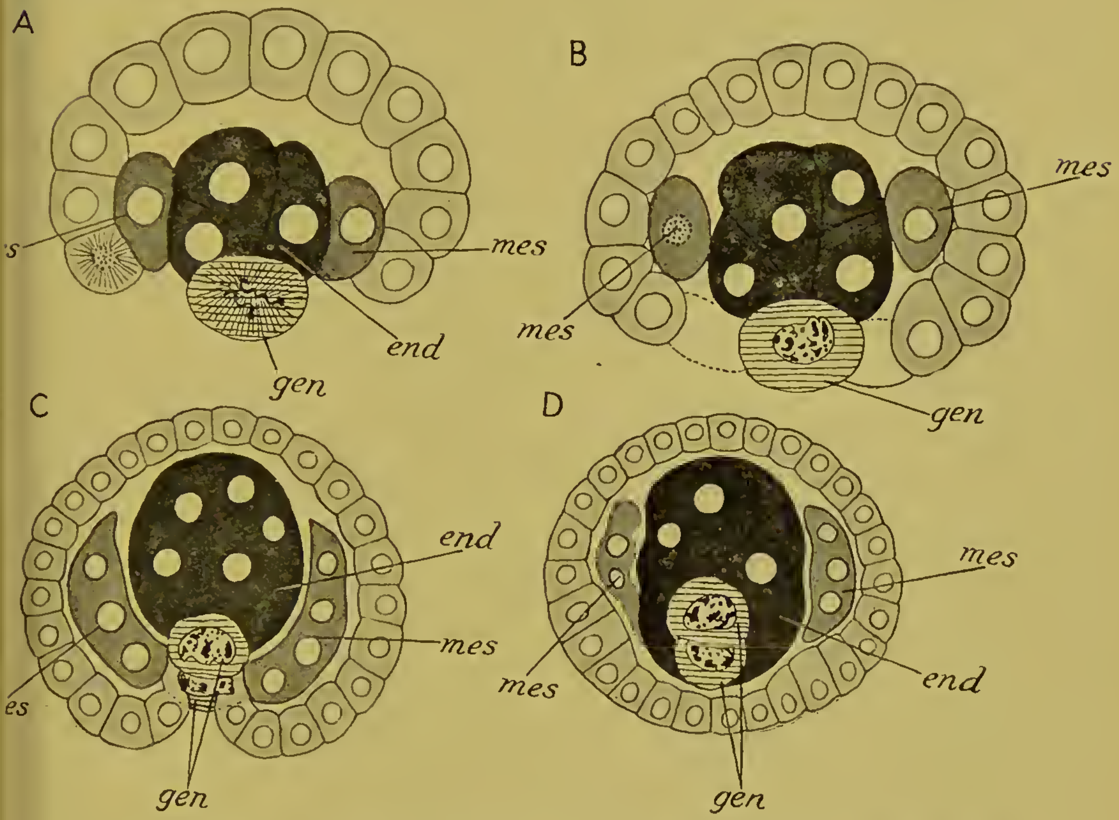

FIG. 351.—Transverse sections of embryo of *Ascaris megalocephala*, in order to show the invagination of the anterior mesoderm cells and of the mother cells of the genital organs. (After Boveri.)

A, section of a stage in which the endoderm cells are invaginated, but in which the mesoderm cells are still superficial. B, section of a stage in which the mesoderm cells have passed into the blastocoelic cavity. C, section of a stage in which the genital cells are beginning to be invaginated. D, section of a stage in which the invagination of the genital cells is complete.

When the young worm finally hatches out—which, under normal circumstances, must occur amongst the horse-dung which has been dropped on the ground—it is known as a **Rhabditis** larva, and it only becomes converted into a mature worm after it has been swallowed by a horse.

The reader will not fail to observe what large gaps there are in the account of the development which has just been given, and what a field there is for future work. The problem of tracing the embryonic rudiments into the larval organs has been most imperfectly accomplished, and it is quite obvious that before much more progress is made in this direction the method of whole mounts will require to be supplemented by that of sections, and some means must be devised for getting the embryos out of the egg-shell.

EXPERIMENTAL EMBRYOLOGY OF NEMATODA

Zur Strassen's descriptive paper, long as it is, pales into insignificance beside his paper on the "T-Giants" (1906). These T-Giants are abnormal embryos produced by the fusion of unfertilized eggs. This fusion occurs only in a very few cases in any one individual, and it is the more apt to occur if the individual is subjected to extreme cold. The eggs first of all cohere, then at the points of contact the egg-shells become softened and finally absorbed, and then their protoplasmic contents are free to coalesce. In this way a multinucleated mass is formed, but usually only one male cell enters it and only one of the egg-nuclei is fertilized; the others degnerate. As a result, an abnormally large egg is formed, and occasionally, if the shape of the egg-shell resulting from the coalescence of several egg-shells is suitable, this large egg may develop into a perfectly normal large embryo.

But in many cases the composite shell is long and narrow, not spherical in shape. Consequently, when the contained composite egg has divided into four and taken on the form of a T (whence the name T-giant), and when in the normal course of things the cell C, which forms the lowest part of the beam of this T, should swing upwards and come into contact with B, the narrowness of the shell prevents it doing so; and after much writhing, which bears witness to the reality of the forces denominated cytotaxis, the cell C settles down to continue its development in its abnormal position.

The life of a T-giant is, however, of very limited duration. The descendants of AB (the dorsal cell family) divide repeatedly, so as to form an irregular vesicle of cells, the ventral cell family carry out several divisions, but soon granular degeneration sets in and the embryo dies. In one case, however, after the first division in the ventral cell family had taken place, the whole embryo had so contracted that it was now possible for C to swing upwards; but this swing upwards took place to the left instead of, as in the normal embryo, in the median plane, and as a consequence C became wedged in between α and β instead of between b and β, as it should have done. Nevertheless, in the further development of this embryo considerable readjustment took place. Although at first the cells of the ventral cell family occupied abnormal positions (*mst*, for instance, being in front of $\mu\sigma\tau$), yet they glided on each other till the normal position was very nearly attained, and, indeed, the embryo showed every sign of developing into a normal larva until it met its death by an accident. In this case it seems as if the abnormal relation of the ventral cell family to the descendants of AB had not seriously affected the development.

Zur Strassen's object in the special study of these T-giants was to elucidate the proximate causes of the orderly succession of all divisions and the orderly movements of the cells on one another, to which the definite form of the body of the embryo owes its origin. Since, even in a T-giant, for instance, C divides evenly into c and γ, which lie side by side where C is widely separated from contact with b and β, it is obvious that the direction of the spindle in this division is in no way influenced, even in the normal embryo, by the neighbourhood of b and β to C. To a certain extent the cell C is self-differentiating, *i.e.* it has the causes of its development within itself, and the question arises whether these causes lie in the cytoplasm or in the nucleus.

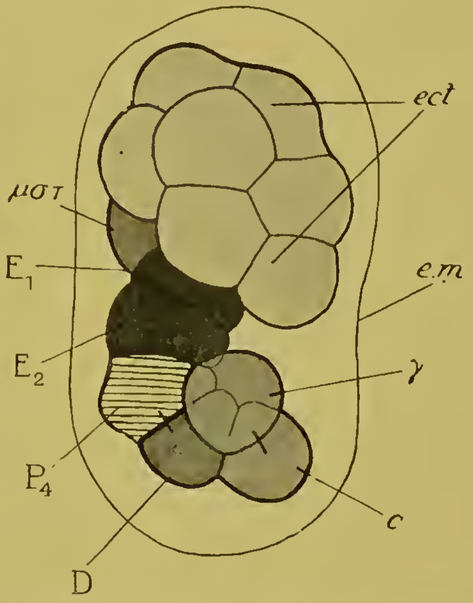

Fig. 352.—A "T-giant" of *Ascaris megalocephala* seen from the left side. (After Zur Strassen.)

ect, ectoderm cells descendants of AB; *e.m*, egg-membrane.

Zur Strassen decides that the causes must lie in the cytoplasm, because the nucleus, both in the resting stage and in the prophases of karyokinesis, is seen to rotate so as to reach its definite position, like a body swept along by a current. The cause he imagines to be an invisible differentiation of the cytoplasm in a definite direction, so that we may conceive its material to be arranged in a series of parallel planes like the cleavage planes of a crystal. These planes, he imagines, compel the spindles of the dividing nuclei to be built up either along them or at right angles to them, and in turn we might suppose that the planes owed their existence to the effects left on the cytoplasm by the previous spindle.

If we do so, Zur Strassen shows, by most acute reasoning, that we are driven back step by step till we have to assume that these planes existed in the fertilized egg. The cytoplasm of the fertilized egg would, on this assumption, be of a most complex constitution. Zur Strassen assumes the existence of three sets of cleavage planes at right angles to each other, as well as of several sets of oblique planes, and there is the possibility that this hypothetical constitution would not suffice if he were to carry his analysis of the development to a later stage.

In order to account for the movements of the cells on one another, which are just as important as the directions of the spindles in determining the form of the embryo, Zur Strassen imagines that these planes are the seats of some sort of attraction which tends to cause similar planes in other cells to set themselves parallel with them. Further, he imagines that this attraction can wax and wane with the physiological condition of the cell, and by a climax of ingenuity he endeavours to account for the swinging round of the beam of the T, first to the left and then into the middle line, by the successive attraction which two sets of planes at right angles to each other, in the cells A and B, exercise on the planes in the lower cell.

The question then arises whether these planes exist in the unfertilized egg or are formed after fertilization. It occasionally happens that a giant egg is doubly fertilized and gives rise to twins. The axes of these twins are often divergent, and sometimes one is much bigger than the other. Hence we have to assume the existences of two complete sets of the hypothetical planes, and to further assume that each set extends through a different region of the cytoplasm. The only way out of the difficulty, according to Zur Strassen, is to assume that the planes *are manufactured in the cytoplasm by influences emanating from the zygote nucleus.*

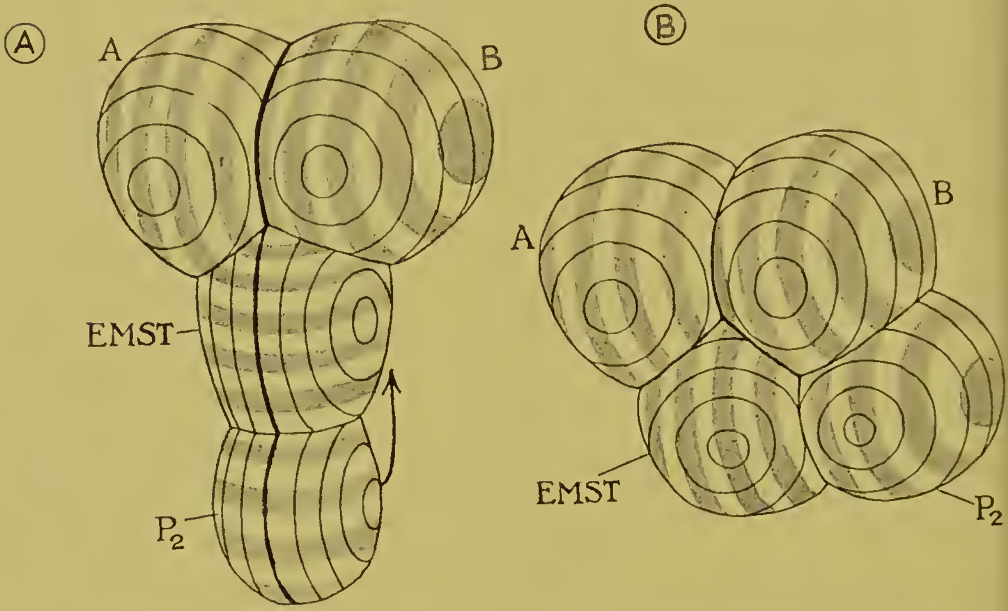

Fig. 353.—Two diagrams of the 4-cell stage in the development of the egg of *Ascaris megalocephala*, in order to show the cytoplasmic zones which Zur Strassen assumes to exist in the blastomeres. A, the T condition. B, the rhomb condition.

One set of planes is indicated by broad bands, another by thin lines. The broad bands in the upper cells first attract the thin lines in the lower cells, and P_2 swings up at right angles to the paper, so that the bands in the upper cells and the lines in the lower are parallel to one another; then the bands in the lower cells are attracted by the bands on the upper cells, and set themselves parallel to these, and so the condition shown in B is attained.

For this assumption there is corroborative evidence afforded by the development of other forms; for instance, in the development of

Dentalium (see p. 325) and still more strongly in the development of the simple Ascidians as worked out by Conklin. If the objection be raised that it is hard to see why if the zygote nucleus has such a profound effect on the cytoplasm its daughter nuclei should not have an equal effect, it must be answered that there is definite evidence in Echinoderm development that they do not have any effect, that, in fact, coincidently with the entrance of the spermatozoon and the union of the two sexual nuclei, a rearrangement of substance takes place which has no parallel in later development. *It is suspected that the separation of ectoderm, endoderm, and mesoderm, which forms the first step in development, the so-called "formation of layers," may find its cause in this rearrangement of cytoplasmic material*; and the well-known circumstance that bud development does not follow the lines of embryonic development (*vide* Chaps. XI., XVII.) may be due to the fact that the nuclei of a bud-rudiment revert to the zygotic condition and rearrange the surrounding cytoplasm but in a different manner.

Whether Zur Strassen's explanations are justified in detail or not, which is very doubtful, it is difficult to escape the cogency of his reasoning that some such differentiation must exist, even in apparently homogeneous cytoplasm, in order to account for the direction of spindles and the movements of cells; unless we are going to have recourse to an imaginary indwelling spirit, the Entelechy of Driesch, to account for these things.

Boveri (1910) has also made investigations on the abnormal embryos of *Ascaris*, but the type of abnormality which he has chosen to investigate is different from that to which Zur Strassen has devoted his attention. Boveri has chosen for his special subject doubly fertilized eggs. These are to be found in small numbers in almost every lot of *Ascaris* eggs submitted to investigation. They are recognized by the fact that at the first cleavage they divide into four equal cells. One of the male nuclei fuses with the female nucleus, and as usual brings in its own "cytocentre" or centrosome, and the other male nucleus forms an independent cytocentre, so that at the first division four cytocentres are formed and the egg divides into four cells.

Now Boveri has proved that there are three varieties of these doubly fertilized embryos. In one variety one of the four cells acts as P, and in subsequent cleavages gives rise to a perfectly normal ventral cell family, whilst the other three act as AB's, and each divides as the upper cell in a normal egg would have done (Fig. 354, A). The product of all three coalesce to form a much too voluminous cell cap of "primary ectoderm," but there are indications that embryos of this type occasionally give rise to normal larvae.

In the second variety two of the four cells act as P's, and two as AB's. The result is the production of a "parallel twin" (Fig. 354, B). Development goes on for a certain distance, but the two ventral cell families interfere with each other's expansion, an irregular mass of cells results, and then death supervenes.

In the third variety only one of the cells functions as AB, the other three act as P's (Fig. 354, C). The three ventral cell families which result become intertwined with one another in the most varied manner. Some cells eventually become detached from one and added to another, and eventually the irregular mass of cells which results dies.

Boveri accounts for the occurrence of these three types of embryo by assuming that the diminution of the chromatin which differentiates AB from P is determined by the presence or absence of some peculiar cytoplasmic substance in part of the egg cell, or rather by its presence in greater amount in one place rather than in another. When three AB's and one P are formed, three of the cytocentres are situated in the region which determines diminution and one in the other region; when three P's and one AB are formed, one of the cytocentres is situated in the region causing diminution and three elsewhere; and, finally, when two AB's are formed, two cytocentres are situated in this region.

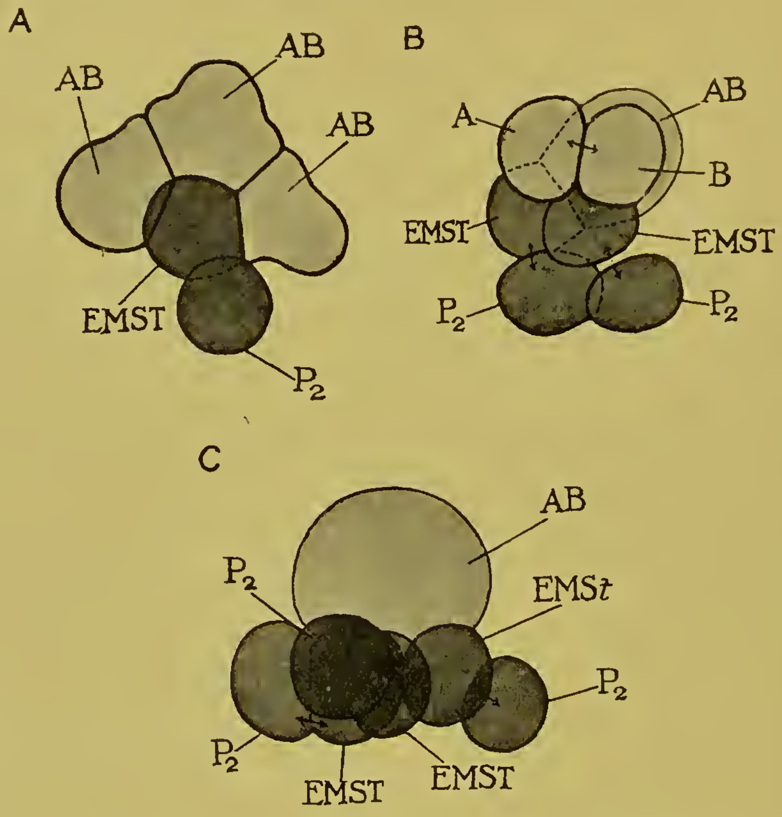

FIG. 354.—The stage corresponding to the 4-cell stage in the development of the normal egg, which occurs in the development of the three types of doubly fertilized eggs of *Ascaris megalocephala*. (After Boveri.)

A, egg in which three dorsal cell families and one ventral cell family develop. B, egg in which two dorsal cell families and two ventral cell families develop. C, egg in which one dorsal cell family and three ventral cell families develop.

In these doubly fertilized eggs a complex spindle is formed connecting all four centres in the most varied and complex ways, and on the parts of this spindle the various chromosomes, derived from the ovum and from the two male cells, are distributed in the most irregular fashion. Boveri, from a collection of suitable cases, shows that the assumption that the process of diminution is a thing determined by the specific character of some of the chromosomes leads to perfectly untenable positions. The conclusion, therefore, that the cause of diminution lies in the cytoplasm is unavoidable.

This conclusion is confirmed by the results of some remarkable experiments which Boveri carried out on the normal eggs of *Ascaris*. When these eggs are subjected to the action of severe centrifugal force (about 3000 revolutions per minute), and when the axis of symmetry of the egg is placed perpendicularly to the axis of rotation, then the first cleavage spindle, instead of lying in the axis of the egg, is situated at right angles to it, *i.e.* tangentially to the direction of rotation, and the egg divides into two precisely equal cells, each of which acts as a P and gives rise to a ventral cell family. The eggs which thus gave rise to two ventral cell families were termed by Boveri **"ball-eggs,"** because a peculiar sphere of granular material, devoid of a nucleus, was found to be ejected from the egg in the direction of a radius of circle of rotation (*ej*, Fig. 355). If the axis of the egg lies obliquely to the axis of rotation, then the first division, even after violent centrifugal action, gives rise to P and AB cells as usual.

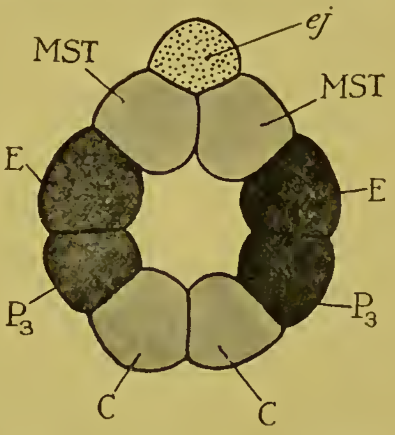

FIG. 355.—Stage in the development of a "ball-egg" of *Ascaris megalocephala* corresponding to the 8-cell stage in the development of a normal egg. (After Boveri.)

ej, ejected mass of granular material.

It follows that, when the peculiar diminution-causing substance is symmetrically distributed with regard to the first spindle, no diminution results, but that when one side of the egg gets a little more than the other, diminution occurs in one of the two daughter nuclei.

That the peculiar development of P and AB is in each case due to the peculiarity of the cytoplasm in each of the two cells, and is not due to the mutual reactions of these cells, is proved by another ingenious experiment of Boveri. A large number of developing *Ascaris* eggs were spread on a slide and covered with a coverslip. On the coverslip a large number of parallel narrow bands of tinfoil were fixed a very short distance apart. When the eggs had reached the 2-cell stage the slide was brightly illuminated by a mercury vapour lamp, the light of which contains a large proportion of ultra-violet rays. These rays kill all the cells they reach, but it often occurred that one cell of a 2-cell embryo was exposed to the light whilst the other was sheltered by the tin-foil. In this way it could be shown that when P is killed AB develops into a closed vesicle of similar cells, and that when AB is killed P gives rise to a typical ventral cell family just as it does in the uninjured egg.

Another most interesting result was obtained by centrifuging the unfertilized eggs. These, under the stress of strong centrifugal force, throw off parts of their substance, so that their volume is often diminished by one-half, and yet when subsequently fertilized they give rise to perfectly normal embryos of correspondingly reduced size.

The conclusion is therefore obvious, that the cytoplasm of the unfertilized egg is homogeneous, and that its differentiation into definite regions only takes place at fertilization, and thus, in a different way, Boveri arrives at exactly the same conclusion as that which Zur Strassen had already reached.

The points sought to be elucidated by Zur Strassen's and Boveri's researches are thus seen to be general questions bearing upon the nature and mechanism of development in general.

The embryonic development of other Nematoda, so far as is known, agrees closely with that of *Ascaris* and ends in the development of a similar Rhabditis larva. The larval development varies enormously according to the condition under which the larval life is passed and the animal or plant in which final ripening takes place; but the study of these life-histories lies outside the scope of this book.

AFFINITIES OF NEMATODA

Before concluding, a few words on the question of the affinities of the Nematoda may be in place. On this question the development throws practically no light at all. Of course, in a general way, it may be said that a hollow blastula is formed and that a gastrula arises by invagination; but the blastomeres are specialized at an unprecedentedly early stage, and yet their specialization is very different from that observed in eggs with spiral cleavage, which are the only other cases where anything like such early specialization is known to occur. Moreover, our knowledge of the complete development is so faulty that we cannot as yet make profitable comparisons. Zur Strassen asserted the existence of a single mesodermic rudiment on each side which, at a stretch, might be compared to a mesodermic band; but his pupil, Müller, asserts that the mesoderm arises from three distinct sources, viz. from MST, from D, and from the most anterior daughters of C.

A thorough and exhaustive revision of the normal development is the first pre-requisite for any profitable theorizing on the subject.

On grounds of comparative anatomy a relationship with the Chaetognatha has been suggested. Both groups have a strong tendency to produce cuticle, both have a simple, straight alimentary canal devoid of appendages, and both possess a single layer of longitudinal muscles and no circular muscles. The excretory organ of Chaetognatha must be regarded as the coelomic cavity. This is suppressed in Nematoda, but a similar phenomenon is seen in Arthropoda, which are undoubtedly derived from the coelomate Annelida, and the suppression of the coelomic cavity may be connected with the excessive production of cuticle. The excretory cells of Nematoda have no known homologues in Chaetognatha and the early development is very dissimilar. That of Chaetognatha is of a very primitive type, whilst that of Nematoda is excessively specialized. In one feature alone do they resemble one another, *i.e.* in the early differentiation of the genital cells.

Perhaps a more thorough knowledge of the normal development of Nematoda would enable us to regard it as a modification of that of Chaetognatha, but for the present the question must be left in abeyance.

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CHAPTER XVI

ECHINODERMATA

Classification adopted—

Eleutherozoa	**Asteroidea**
	Ophiuroidea
	Echinoidea
	Holothuroidea
Pelmatozoa	**Blastoidea (Extinct)**
	Cystoidea (Extinct)
	Thecoidea (Extinct)
	Crinoidea

THE Echinodermata, as they at present exist, are divided into five very distinct classes of animals, viz. **Asteroidea**, **Ophiuroidea**, **Echinoidea**, **Holothuroidea**, and **Crinoidea**. The first four of these classes agree in possessing the power of free locomotion during the adult stage; and, when a fixed stage does occur in the course of development, the fixing organ is attached to the oral surface of the animal. For this reason the four classes mentioned are grouped together in a sub-phylum termed **Eleutherozoa**. The members of the fifth class, Crinoidea, possess at some time of their existence a fixing organ situated at the aboral pole of the body, and this organ is retained by many of them throughout life; for this reason the Crinoidea, together with some extinct classes of Echinodermata, are grouped together as **Pelmatozoa**.

In three of the four classes of Eleutherozoa the development of one species has been worked out in great detail, and in the case of the fourth class, although many points are still obscure, the general course of the development is well known. The development of only one Pelmatozoon is known, and in this case a large part of the development is passed through within the egg-membrane; whereas the corresponding stages of development in Eleutherozoa are passed through in the free larval condition.

A great deal of experimental work has been done on the eggs and larvae of Echinodermata, and most important results have been obtained which differ in many points from the results obtained from experiments on the eggs of those groups of animals (*cf.* Coelenterata, Nemertinea, Mollusca, and Nematoda), which we have already discussed. For this reason it is necessary that the normal course of development in Echinodermata should be described in detail.

Of the four groups of Eleutherozoa, the one which presents the most primitive features in its development is the Asteroidea, and we shall commence our study of the development of Echinodermata by a consideration of this group.

ASTEROIDEA

In a few cases the entire development of an Asteroid has been worked out, and the manner in which the organs of the adult are fashioned out of the body of the larva has been elucidated. The species which have been thus studied are *Asterina gibbosa*, *Cribrella oculata*, and *Solaster endeca*. Unfortunately in all these cases we have to deal with an egg which contains a good deal of yolk, and with a development which is much modified and hurried on. *Asterina gibbosa* is the least modified of the three, for its larva possesses a mouth and it can take food in the larval condition, whereas in the case of the other two species the gut is delayed in its development, and the larva possesses no mouth.

In the more normal type of development, such as is met with in the genera *Asterias*, *Astropecten*, *Luidia*, etc., the egg develops into a larva which possesses a complete alimentary canal, and lives a free-swimming and self-supporting life for a long period, often extending over two months, until at last it metamorphoses into the adult form. The eggs of several species of *Asterias* have been successfully reared through the metamorphosis by feeding them with cultures of various forms of diatom.

A complete account of the development of *Asterias rubens* by Dr. Gemmill is now in the press, and will shortly be available to all zoologists; by Dr. Gemmill's kindness, however, we are allowed to make use of some of his results which are as yet unpublished. Field (1894) has given an account of the segmentation of the egg and of the early larval stages in the development of the American species (*Asterias vulgaris*). We have also an account of the full-grown larva, and of the metamorphosis of *Asterias pallida*, by Dr. Goto (1897). Now *Asterias pallida* is a mere synonym for *Asterias vulgaris*, and this starfish is considered by systematists to be very closely allied to *Asterias rubens*, the common British starfish, of which some indeed consider it to be a local variety. By piecing together, therefore, Field's, Gemmill's, and Goto's observations a fairly complete story can be made out, and it is important to notice that in most important points Goto's results demonstrate that, in so far as the building up of the adult organs is concerned, *Asterias pallida* agrees very closely with *Asterina gibbosa*. As the organogeny of the latter species has been very thoroughly worked out we can thus use the data derived from it, with some confidence, in filling in the gaps in our story of asteroid development.

Before entering on this subject, however, there are certain practical questions to be considered. Loeb, in his work *Die chemische Entwicklung des tierischen Eies* (1910), states that the eggs of *Asterias* are not ripe when laid, but ripen after lying in sea-water for five or six hours. This seems to be a most misleading statement. The eggs when shaken out of the excised ovary, or when made to exude through the genital openings by the application of pressure, are certainly unripe. They are surrounded by a glassy chorion which disappears in a few hours and renders them fit to receive the spermatozoa. But if perfectly ripe males and females be selected, they will often emit their genital products spontaneously, and then every egg which is emitted is capable of instant fertilization.

As to methods of preservation and of preparation a few words may be said here. Goto used corrosive sublimate, with a certain proportion of glycerine and of acetic acid, to preserve the larvae, but it is exceedingly doubtful whether his histological results are at all reliable. Further, this preserving fluid has one strong disadvantage, it is strongly acid, and, as a developing Echinoderm contains numerous calcareous deposits, the sudden solution of these is apt to generate quantities of carbonic acid gas, which distend and tear the tissues. The cavities so produced, which even so great an authority as Ludwig (1882) has regarded as existing in the living animal, are nevertheless only artefacts.

Faced with this difficulty, we ourselves, many years ago, adopted a method of preservation which, although it has numerous drawbacks, still yields better results with Echinoderm larvae than any other. This method consists in adding a half per cent or even a quarter per cent solution of osmium tetroxide to the watch-glass containing the eggs and larvae, and leaving them in the mixture till they are thoroughly impregnated with it and have assumed an almost black colour. They are then transferred to a bottle containing Müller's fluid (which is a mixture of the solutions of bichromate of potash and sulphate of soda), in which they remain for twenty-four hours at least, though several months' immersion in this fluid does not hurt them. After treatment with Müller's fluid they are rinsed in distilled water, and then dehydrated by being passed successively through various grades of alcohol. They are embedded in celloidin and then in paraffin, according to the method described in Chapter II. They may or may not be stained in borax-carmine before being embedded, for the effect of this is merely to darken their colour and render them more easy to orientate before finally making up the blocks of paraffin. In all cases the sections are finally stained on the slide in Grenacher's haematoxylin.

The penetration with osmium tetroxide gives a black stain of a diffuse kind; this is, in fact, due to a deposit of the metal osmium in the cells, and it is difficult under these circumstances to get the ordinary staining agents to act on tissues so impregnated. It is found in practice that if the sections be immersed in a strong solution of borax-carmine for twenty-four hours they absorb little or no stain; if, however, after this treatment they are transferred to a dilute-solution of Grenacher's haematoxylin in distilled water they will become densely stained.

The advantages of this method are, (1) that the unsaturated chromic acid in the bichromate of potash acts on calcareous deposits so slowly and gently that they are dissolved without any accumulation of carbonic acid gas resulting, and without any tearing of the surrounding tissues, because such carbonic acid as is produced is dissolved in the water of the solution as fast as it is produced; (2) that the form of the cells and tissues is preserved with the most exquisite faithfulness, such delicate structures as fine flagella being clearly visible when an immersion lens is used.

If a section of a larva preserved in osmium tetroxide and Müller's fluid be compared with a section of one preserved in corrosive sublimate it will be seen that in the latter there is what one might almost term a clotted appearance, *i.e.* the delicate cells of the connective tissue tend to cohere in lumps, and the cytoplasm is seen, when subjected to minute examination, to be somewhat shrunken, and to stain badly; whereas in the osmium tetroxide preparation the individual forms of these connective tissue cells are exquisitely preserved, and their cytoplasm is deeply stained and sharply distinguished from the semi-fluid ground substance in which they are immersed.

The disadvantages of the method are, (1) that it renders the objects so treated very brittle, and hence arises the absolute necessity of embedding in celloidin. For this reason it is less suitable for dealing with yolky eggs, like those of *Cribrella* and *Solaster*, than with comparative yolkless eggs, like those of *Asterias*, since the combination of the osmium tetroxide with the particles of yolk is extremely hard and resisting to the knife; (2) if applied to embryos of any size the osmium tetroxide is apt to form a hard black crust on the outside, and to prevent the penetration of the fixing fluid to the interior of the specimen, which is thus badly preserved. For this reason we have sometimes used a dilute solution of osmium tetroxide (·25 per cent), and then allowed it to act for a considerable time, because the fixative is thus enabled to penetrate to the interior.

For the preservation of the delicate pelagic larvae which occur in the life-histories of the least modified Echinoderms no other method gives results of equal value to this one. The difficulty of brittleness can be got over by the use of the celloidin-paraffin method of embedding, by good razors, and by restricting the period of embedding in the hot paraffin as much as possible. For yolky embryos and larvae, like those of *Solaster*, the mixture of corrosive sublimate and acetic acid, in spite of its disadvantages, will probably give the best results on the whole.

For making whole mounts other means must be adopted. Good results may be attained by the following method:—The larvae are deluged with the strongest formalin (40 per cent solution); this is neutralized before being used by having a piece of chalk immersed in it. The formalin is only allowed to act for a couple of minutes, and the larvae are at once transferred to absolute alcohol, to which a drop of strong ammonia has been added. They are then stained in a solution of either eosin or safranin in absolute alcohol, which must be allowed to act for at least twenty-four hours, preferably for several days. Then, drop by drop, at considerable intervals, oil of cloves is added,—it is best to prolong the period of the addition of oil of cloves over several days,—and then the larvae are transferred to pure oil of cloves for several days. Finally, they are placed in the well of a concave slide and a drop of a thick solution of Canada balsam in xylol is placed upon them; the oil of cloves flies off by surface tension to the periphery, and can be—if the operation is skilfully performed—almost entirely removed by blotting-paper. A coverslip is now gradually pushed over the preparation from the side, so as to avoid the formation of bubbles, and in this way a permanent preparation is made.

For younger stages, where there is not so much gelatinous tissue as in the older larvae, a simpler method has been invented by Professor Graham Kerr. He fixes them by immersing them (when freed from as much of the salt water which clings to them as possible) in absolute alcohol. They are stained in safranin dissolved in absolute alcohol, and are then mounted in *balsam dissolved in absolute alcohol.* Only a thin solution of this can be obtained, and it shrinks greatly in the drying, but by patient addition of fresh solution, as that which is just added dries, very beautiful permanent mounts may be obtained.

ASTERIAS

The egg of *Asterias* segments with great regularity into blastomeres of approximately equal size. It is a beautiful example of indeterminate segmentation. The result of segmentation is a hollow **blastula**, and it can be shown experimentally that, up to the 500-cell stage, this blastula is not functionally specialized in any way, but that any sufficiently large fragment cut from it will heal up by the approximation of its edges, and so form a miniature blastula which will develop into a perfect miniature larva. At about the 1000-cell stage the cells develop cilia, and the blastula begins to rotate within the egg-membrane, which it soon bursts, and it then rises to the surface of the water and begins its existence as a free-swimming larva. Certain of the Porifera and of the Coelenterata are the only species of animals, outside the phylum Echinodermata, in which the larval existence is begun as early as it is in the development of *Asterias.*

At the end of a day of free-swimming life the blastula begins to be converted into a **gastrula**. The blastula loses its spherical shape and becomes flattened on one side, which we shall term the posterior side of the larva. In the middle of this flattened surface an invagination makes its appearance which is the beginning of the **archenteron.** This invagination is of small diameter compared with the diameter of the larva, and, in contradistinction to all the gastrulae so far studied, a wide space, the **primary body-cavity** or **blastocoele,** intervenes between endoderm and ectoderm. In most gastrulae a slit-like blastocoele is present, but in *Asterias* it is enormous.

As the invagination progresses—or, according to Field, from its very beginning—cells are budded off from the invaginating surface into the blastocoele. These cells are termed **mesenchyme.** The "wandering" of these cells seems to be effected by their emitting long filamentous pseudopodia which span the blastocoele, and along these strands the body of the cell glides like a drop of dew on a spider's web. From the mesenchyme an exceedingly delicate gelatinous connective tissue is formed, since the mesenchyme cells and their pseudopodia secrete a few intercrossing fibres; but the fluid "ground-substance," which from the beginning has occupied the cavity of this blastocoele, forms the great mass of the "tissue" until the completion of metamorphosis.

As the invagination proceeds the gastrula grows in length, changing its shape from a hemispherical to a cylindrical form, and when the archenteron has attained about two-thirds the length of the larva, it develops at its end a thin-walled vesicle, and the process of gastrulation may be said to be complete. From this vesicle mesenchyme cells continue to be budded off. The ectoderm cells at the anterior pole of the larva become rather more columnar than elsewhere (*ap*, Fig. 356, C), and bear longer cilia. This thickening we may regard as a rudimentary sensory **apical plate,** but no nerve-fibres have as yet been detected at the base of these cells.

In *Asterias rubens,* according to Gemmill, the formation of mesenchyme does not begin so early as in *Asterias vulgaris,* no mesenchyme at all being formed in the British species until invagination is well advanced.

From the vesicle at the apex of the archenteron two lateral pockets grow out (Fig. 356, D). These are the rudiments of the **coelomic sacs,** and they soon become completely cut off from the archenteron, which in this way becomes divided into **coelom** and **gut.** It is not clear, from the accounts which we possess, whether the two coelomic sacs are, or are not, at first united across the middle line by the remains of the original vesicle; the coelomic sacs certainly are united in this way in the larvae of Ophiuroidea and Echinoidea. From the walls of the coelomic sacs more mesenchyme cells are given off. The pseudopodia of these cells, which, as we have seen, span the blastocoele, become in many cases muscular, and confer on the larva powers of bending and of changing its shape.

The **anus** is nothing but the persistent opening of the **blastopore,** but the **mouth** is the external opening of a wide funnel formed by an ectodermic invagination situated at about one-third the length of the larva from its front end. This invagination is of course the **stomodaeum.** Before the stomodaeum meets the gut, the latter becomes divided by two constrictions into three regions. Of these regions the hindermost is the **intestine**, the middle one becomes globular and forms the **stomach**, whilst the most anterior forms the endodermal portion of the **oesophagus** which meets and fuses with the stomodaeum (Fig. 357, B), and thus completes the larval oesophagus. Along the sides of this oesophagus a V-shaped band of strongly ciliated epithelium is differentiated, which is termed the **adoral ciliated band** (Fig. 357, C). It seems to be formed from both the ectodermal and the endodermal region of the oesophagus. The angle of the V is situated behind in the mid-ventral line. The limbs of the V pass up the sides of the oesophagus, and their terminations are

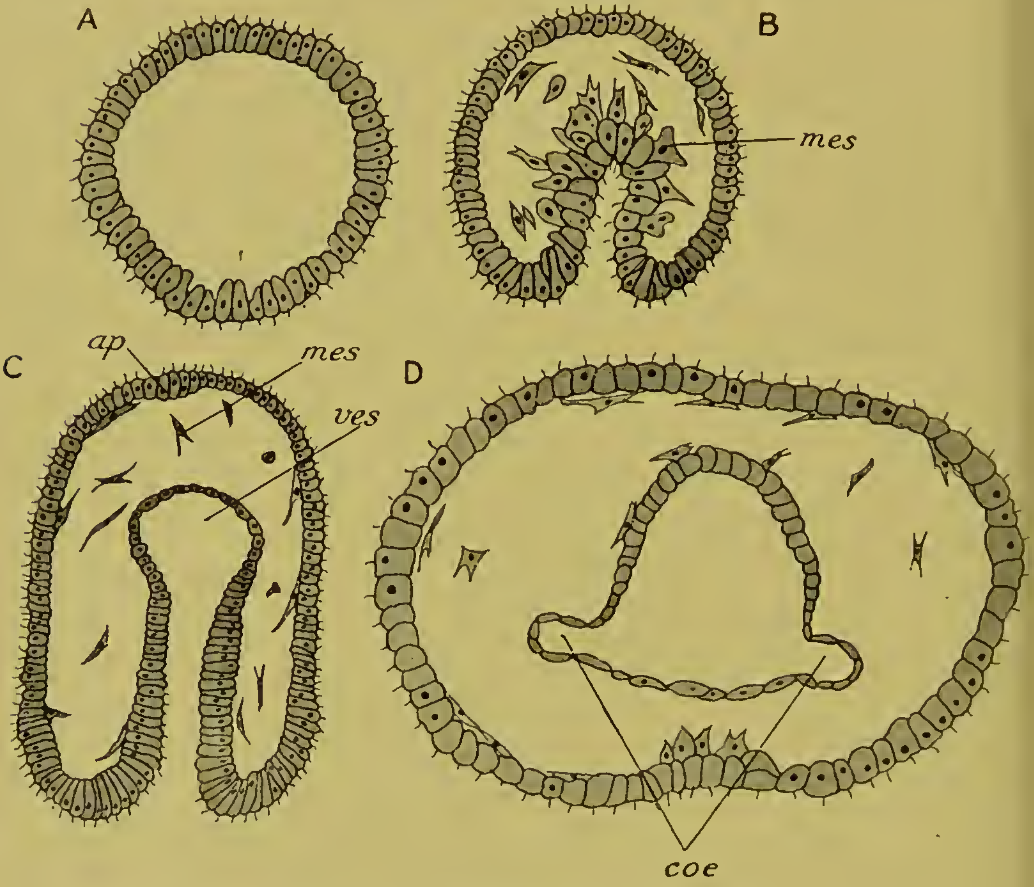

Fig. 356.—Stages in the early development of *Asterias vulgaris*. (After Field.)

A, blastula in optical longitudinal section. B, gastrula in optical longitudinal section showing the formation of mesenchyme. C, older gastrula in optical longitudinal section. D, transverse section of a larva slightly older than that represented in C, showing the formation of the coelom. *ap*, apical disc; *coe*, coelomic sac originating as pouches from the archenteron; *mes*, mesenchyme; *ves*, vesicle at the apex of the archenteron.

connected by a much less strongly ciliated band, which passes round the dorsal side of the oesophagus just behind the mouth.

It is commonly taken for granted that the function of the adoral band is to direct a stream of water carrying minute organisms into the mouth, and that it is in this way that the larva secures its nourishment. Some years ago we made some observations on the function of the homologous band in the larva of *Echinus*, and it seems to us that the main function of the adoral ciliated band, like the function of the cilia in the transverse grooves running across the labial palps of Pelecypod Mollusca, is to *remove* excess of food from the neighbourhood of the mouth. The minute organisms, which constitute the bulk of the food, may be seen to be carried in by a current which passes into the stomodaeum at its dorsal border. This current seems to be caused by the cilia of the principal longitudinal band (*v. infra*), aided no doubt by the cilia of the dorsal side of the stomodaeum. At the ventral end of the stomodaeum particles may be seen to be flung outwards violently—hence it is apparent that the current produced by the adoral band is directed outwards. The food accumulated in the outer end of the stomodaeum is transferred to the stomach, not by the action of cilia but by peristaltic muscular contractions.

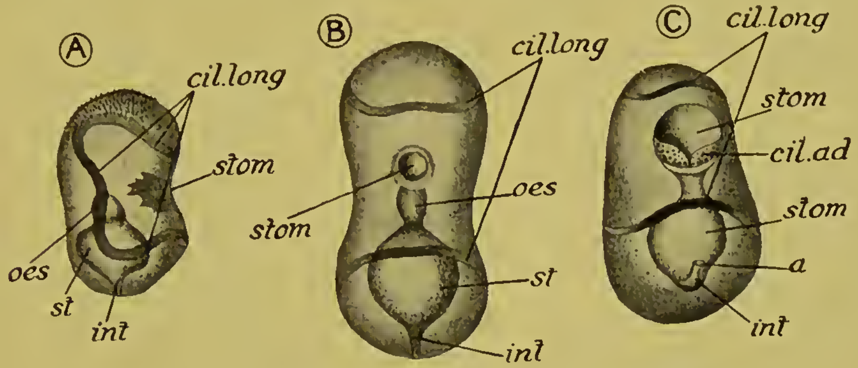

FIG. 357.—Young larvae of *Asterias vulgaris*. (After Field.)

A, about three days old, from the side. B, about four days old, from the ventral surface. C, about five days old, from the ventral side. *a*, anus; *cil.ad*, adoral band of cilia; *cil.long*, longitudinal ciliated band; *int*, intestine; *oes*, oesophagus; *st*, stomach; *stom*, stomodaeum.

Whilst these changes have been taking place other events have been occurring. The cilia, which covered the whole surface of the blastula and gastrula, become specially abundant and long over the course of a sinuous band of thickened epithelium which is termed the **longitudinal ciliated band** (*cil.long*, Fig. 357, A), and which is the principal locomotor organ of the larva. Over the rest of the surface they do not disappear, but become very sparse. This is due to the passive stretching of the epithelial cells in these regions, due to the increase in the pressure of the blastocoelic fluid.

The **longitudinal ciliated band** is also found in the larvae of

Ophiuroidea, Echinoidea, and Holothuroidea. It consists of two sides, and of an anterior and of a posterior cross-bar. The anterior cross-bar is situated in front of the mouth, and the posterior cross-bar in front of the anus. Neither cross-bar is straight, both are bent into the form of loops. The loop formed by the anterior cross-bar is bent back along the ventral surface of the prae-oral region or forehead of the larva, and it is termed the **prae-oral loop**, and the area which it surrounds is termed the **frontal field**. The loop formed by the posterior cross-bar bends forward along the ventral surface in front of the anus, and the area which it surrounds is called the **anal field**.

When the alimentary canal has been completed by the union of the stomodaeum and oesophagus the larva is able to feed, and, if suitable diatoms be provided, it will live and grow, even in a comparatively small aquarium, without any change of water. Dr. Gemmill has reared the larvae of *Asterias rubens* for over two months in his laboratory at Glasgow, until they had completed their metamorphoses; and this feat has also been accomplished with the larvae of *Asterias glacialis* by Professor Yves Delage in his laboratory at Roscoff.

As the larva increases in size the tissue of the longitudinal ciliated band grows more quickly than adjacent regions of the ectoderm, and the band becomes thrown into folds. These folds form lobe-like outgrowths termed **larval arms**, which correspond in number, position, and size on the two sides of the body, and confer on the larva a "bipinnate" appearance, whence the name **Bipinnaria**. Mortensen (1898) has invented a nomenclature for the larval arms of Asteroidea and the homologous structures in other Echinoderm larvae, and we shall follow his nomenclature in this book.

Before the larval arms have attained any size the prae-oral loop becomes separated from the rest of the longitudinal ciliated band, and this primary band becomes, in this way, divided into two secondary bands, which we shall term the **prae-oral** and the **post-oral bands** respectively (*pr.o.b, p.o.b,* Fig. 358).

In the Bipinnaria larva of *Asterias vulgaris* the prae-oral band carries only two larval arms, which are termed the **prae-oral arms** (*pr.o.a,* Figs. 358, 359), and the frontal field is somewhat quadrangular in shape. But in the larvae of the European species, *Asterias rubens* and *A. glacialis,* the prae-oral band develops in addition a median anterior arm, directed forwards, termed the **median ventral arm**, and the frontal field is consequently triangular in shape. From the post-oral band, in all three species of *Asterias,* there is developed an anteriorly directed arm, which is termed the **median dorsal arm** (*m.d.a,* Fig. 358). From the sides of the post-oral band, about one-third the length of the larva from its anterior end, there are given off two arms, termed the **antero-dorsal arms** (*a.d.a,* Fig. 358). Still farther back, at rather more than two-thirds the length of the larva from its anterior end, two similar arms arise termed the **postero-**

dorsal arms (*p.d.a,* Fig. 358). Where the sides of the post-oral band pass into the anal loop two long arms are developed, termed the **postero-lateral arms** (*p.l.a,* Fig. 358). Finally, from the sides of the anal loop two short arms are developed, termed the **post-oral arms** (*p.o.a,* Fig. 358).

The two coelomic sacs are, for a considerable time, two somewhat rounded pockets lying at the sides of the oesophagus. The one on the left side sends up a short vertical outgrowth which fuses with a slight inward dip of the ectoderm, and in this way forms a canal leading to the exterior. The opening of this canal is termed the **primary madreporic pore** (*m.p,* Fig. 359), and the canal itself is termed the **pore-canal.** Its wall is covered with cilia which beat inwards and strive to distend the coelomic sac with sea-water.

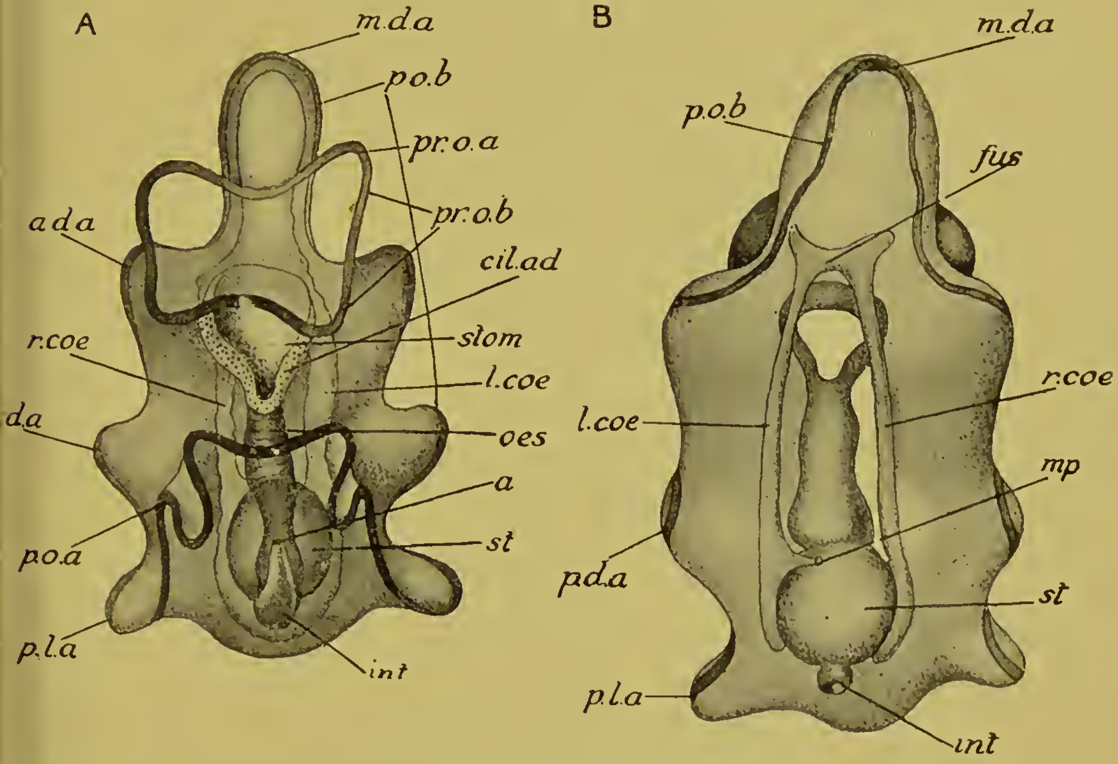

Fig. 358.—Fully developed Bipinnaria larva of *Asterias vulgaris,* about three weeks old. (After Field.)

A, from the ventral surface. B, from the dorsal surface. *a,* anus; *a.d.a,* anterior dorsal arm; *cil.ad,* adoral ciliated band; *fus,* anterior fusion of right and left coelomic sac; *int,* intestine; *l.coe,* left coelomic sac; *m.d.a,* median dorsal arm; *m.p,* madreporic pore; *oes,* oesophagus; *p.d.a,* postero-dorsal arm; *p.l.a,* postero-lateral arm; *p.o.a,* post-oral arm; *p.o.b,* post-oral band of cilia; *pr.o.a,* prae-oral arm; *pr.o.b,* prae-oral band of cilia; *r.coe,* right coelomic sac; *st,* stomach; *stom,* stomodaeum.

Twenty years ago Field (1894) stated that, in the case of *Asterias vulgaris,* the right coelomic sac formed a similar pore-canal, terminating in a **right madreporic pore** (m^1p^1, Fig. 359), which soon, however, became obliterated. No subsequent observer recorded the existence of this right madreporic pore, although the larvae of *Asterias* were raised by thousands for experimental purposes by Driesch, Herbst, and others; but quite recently Dr. Gemmill has been able to confirm Field's statement. He finds that a right madreporic pore is formed in about one in every ten larvae of *Asterias rubens,* and in one out of every two larvae of *Asterias glacialis.* In all cases it very soon closes.

The appearance of a right and left madreporic pore is the first indication of what is really the key to the understanding of Echinoderm development, viz. the fact that the two sides of the larva originally gave rise to precisely similar organs, but that some of these organs grew and developed on the left side while they atrophied on the right, and that thus an asymmetry was produced.

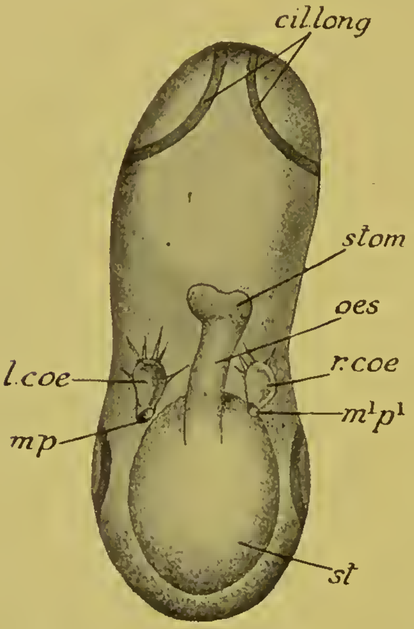

FIG. 359.—Larva of *Asterias vulgaris* four days old, viewed from the dorsal surface, showing two madreporic pores. (After Field.)

Names as in the preceding figure. In addition, *l.coe,* left coelomic sac; *m.p* (left), persistent madreporic pore; m^1p^1 (right), transitory madreporic pore; *r.coe,* right coelomic sac.

The coelomic sacs now begin to grow in length until they form long, narrow, cylindrical cavities, reaching from the prae-oral region of the larva to the posterior end; and by their form and relations they merit the name "**water-tube,**" bestowed on them by Agassiz (1864). The right and left water-tubes meet one another in the prae-oral lobe and fuse into one (*fus,* Fig. 358, B), but elsewhere they remain separated from one another by the alimentary canal, and above and below this by a vertical mesentery.

Now a constriction appears just behind the madreporic pore, which almost, but not quite, divides the left water-tube into anterior and posterior portions. On the right side no such constriction is formed until considerably later. The front division of the left water-tube may be termed the **left anterior coelom,** whilst the hinder division is named the **left posterior coelom** (*l.p.c,* Fig. 360, A). The posterior portion of the left anterior coelom swells out slightly, and begins to form five lobe-like outgrowths arranged in an open curve. This swelling and its lobes are the rudiment of the water vascular system, and are termed the **hydrocoele** (*hy,* Fig. 360). The most dorsally situated lobe is numbered (1), the next (2), and so on. Occasionally a similar five-lobed outgrowth, which we may term the **right hydrocoele,** is formed as an outgrowth from the right water-tube. In this case this water-tube becomes completely divided into anterior and posterior portions, which we may term **right anterior** and **right posterior coeloms** respectively, just as the left is normally. This formation of a second hydrocoele was first described by us in *Asterina* (1896).

Somewhat later, near the mid-dorsal line but to the right of it, a small sac is formed. According to Field (1894) it just appears as a solid bud of cells in the blastocoele, but in *Asterina gibbosa* (Fig. 365) it is certainly budded off from the posterior wall of the right side of the anterior coelom, with which it remains connected for some time by a solid string of cells. The bud is, from the first, nearly but not quite solid; it would be correctly described as a very thick-walled evagination of the right anterior coelom. It is soon cut off from the anterior coelom, and then its cavity rapidly enlarges and it becomes thin-walled.

In *Asterias* Goto (1897) has seen this cavity connected by a string of cells with the *left* anterior coelom, but it is practically certain that a re-examination of this point will show that *Asterias* and *Asterina* agree in essentials. Gote's observations are exceedingly incomplete, and it is by no means clear that the "scattered string of cells," which he saw connecting the sac with the left anterior coelom, represents the original connection of the sac with the coelom.

This sac may be termed the **madreporic vesicle**. According to Gemmill it executes slow pulsations. It may be compared to the pericardial vesicle of *Balanoglossus* (see p. 575). We formerly (1896) regarded the madreporic vesicle as the vestigial right hydrocoele, but the observations of Gemmill, who has seen this vesicle and a well-developed right hydrocoele present in the same larva, render this view untenable.

METAMORPHOSIS OF ASTERIAS

With the formation of the madreporic vesicle, and of the rudiment of the water vascular system, the Bipinnaria has reached the summit of its development as a free-swimming organism. It now begins to prepare to take up a fixed life, and with this change in habits the metamorphosis may be said to begin. From the anterior end of the larva, between the prae-oral and post-oral bands, there grow out three clubbed arms which are not ciliated in the larva of *A. vulgaris*, but in the larva of *A. rubens* and of *A. glacialis* the median ventral "arm" of the prae-oral ciliated band is continued on to them. These arms contain diverticula of the anterior coelom. One is median and dorsal (*br.med*, Fig. 360), and the other two are situated symmetrically to the right and left of it (*br.lat*, Fig. 362). These processes are termed the **brachiolar arms**, and the larva is now termed a **Brachiolaria**. It still swims, but it occasionally attaches itself to the side of the vessel in which it is contained by the brachiolar arms, which apparently act as suckers. Goto states that the cells forming the walls of the coelomic vesicles develop muscular fibrils at their bases, which are for the most part disposed circularly, but some of which pursue a longitudinal course. Now the brachiolarian arms differ from the other larval arms in possessing hollow outgrowths from the coelom within them, and it appears certain that the longitudinal muscular fibres accompanying these outgrowths can cause a retraction of the central portions of the tips of the brachiolarian arms, and thus enable them to act as suckers.

As we have noted above, the right and left coelomic sacs fuse with one another in the prae-oral lobe, and the left becomes almost divided into two by a constriction which appears just behind the hydrocoele. Somewhat later a similar constriction appears in the right coelomic sac, and by these two constrictions two posterior regions, the **left posterior coelom** and the **right posterior coelom**, become marked off from the left and right coelomic sacs. The left posterior coelom begins to extend beneath the gut over to the right side; this extension is known as the **right ventral horn** of the left posterior coelom, and its formation causes the whole sac to take on the form of a U (Fig. 361). It fuses with the right coelomic sac in *front of the constriction*, separating it into anterior and posterior portions—in a word, it fuses with the right anterior coelom. Soon, left and right anterior coelomic sacs, already fused in front, become completely merged in one another so as to form a single **anterior coelom.** The right

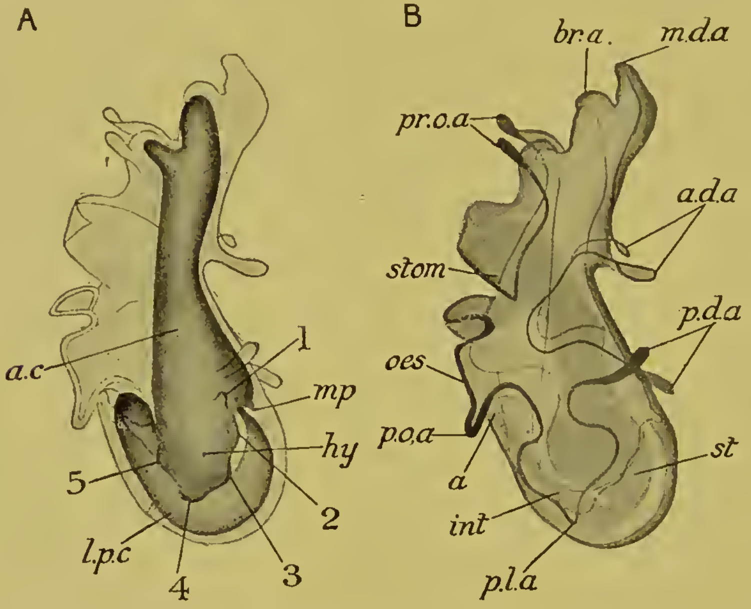

Fig. 360.—Lateral views of an advanced Bipinnaria of *Asterias vulgaris*, in which the brachiolarian arms are just appearing. (After Goto.)

A, outline view to show the segmentation of the coelom. B, more detailed sketch. Letters as in Fig. 358. In addition, *a.c*, anterior coelom; *br.a*, rudiment of anterior median brachiolarian arm; *hy*, hydrocoele; 1-5, the five lobes of the hydrocoele; *l.p.c*, left posterior coelom.

posterior coelom becomes entirely cut off from the anterior coelom: it is termed the **epigastric coelom** by Goto, who thought that it was formed by the growth of an independent longitudinal septum, but this error has been corrected by Gemmill.

By the time that these changes have been accomplished the brachiolar arms have been formed; and in the centre of the circle formed by them a circular disc of thickened glandular ectoderm appears. This is the organ for permanent fixation (*fix*, Fig. 362). Holding on by its brachiolar arms the larva brings this disc into close contact with the substratum and *thus permanently fixes itself.*

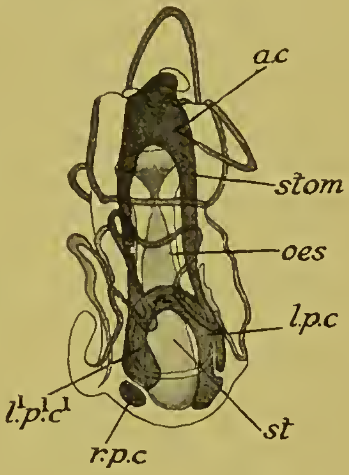

FIG. 361. — Ventral view of a Bipinnaria of *Asterias vulgaris* of the same age as that shown in Fig. 360, in order to show the mutual relations of the coelomic cavities. (After Goto.)

Letters as in preceding figure. In addition, $l^1p^1c^1$, right ventral horn of left posterior coelom; *r.p.c*, right posterior coelom.

The larva may now be said to be differentiated into a posterior region containing the stomach, the intestine, the hydrocoele, and the right and left posterior coeloms; and into an anterior region, consisting of the prae-oral lobe, containing the mouth, oesophagus, and the anterior coelom. The anterior region may be termed the **stalk,** the more posterior the **disc.** Once the larva has become firmly attached the stalk is progressively shortened (Fig. 362, B).

The **stone-canal** makes its appearance as an open groove of ciliated epithelium, situated on the anterior aspect of the septum dividing anterior from left posterior coeloms. It begins just beneath the inner end of the pore-canal, and it runs down to the spot where the anterior coelom is beginning to be pinched from the hydrocoele.

On the posterior aspect of the septum there appears a groove-like outgrowth of the left posterior coelom. This groove is the rudiment of the **peri-oral** coelom. It grows into a tube which extends in the form of a slight crescent beneath a faint bulge of the stomach, which is the rudiment of the **adult stomach.**

The stomodaeum becomes disconnected from the endodermal portion of the oesophagus: it persists for a brief time as a shallow pit of the ectoderm, but eventually disappears entirely, and the oesophagus becomes, as metamorphosis proceeds, a less and less conspicuous appendage of the stomach. The anus of the larva is also obliterated, and the intestine becomes shortened till it forms a very short tag attached to the stomach. According to Gemmill this tag persists throughout metamorphosis, and from it the **rectum** of the adult is developed.

Five thickened lobes now appear on the ectoderm covering the right posterior coelom. These are the first traces of the **arms** of the future star-fish and are termed collectively the **aboral disc**. As the ventral horn of the left posterior coelom extends further and further round to the right, and grows in size, the right posterior coelom and the disc covering it become displaced backwards, and

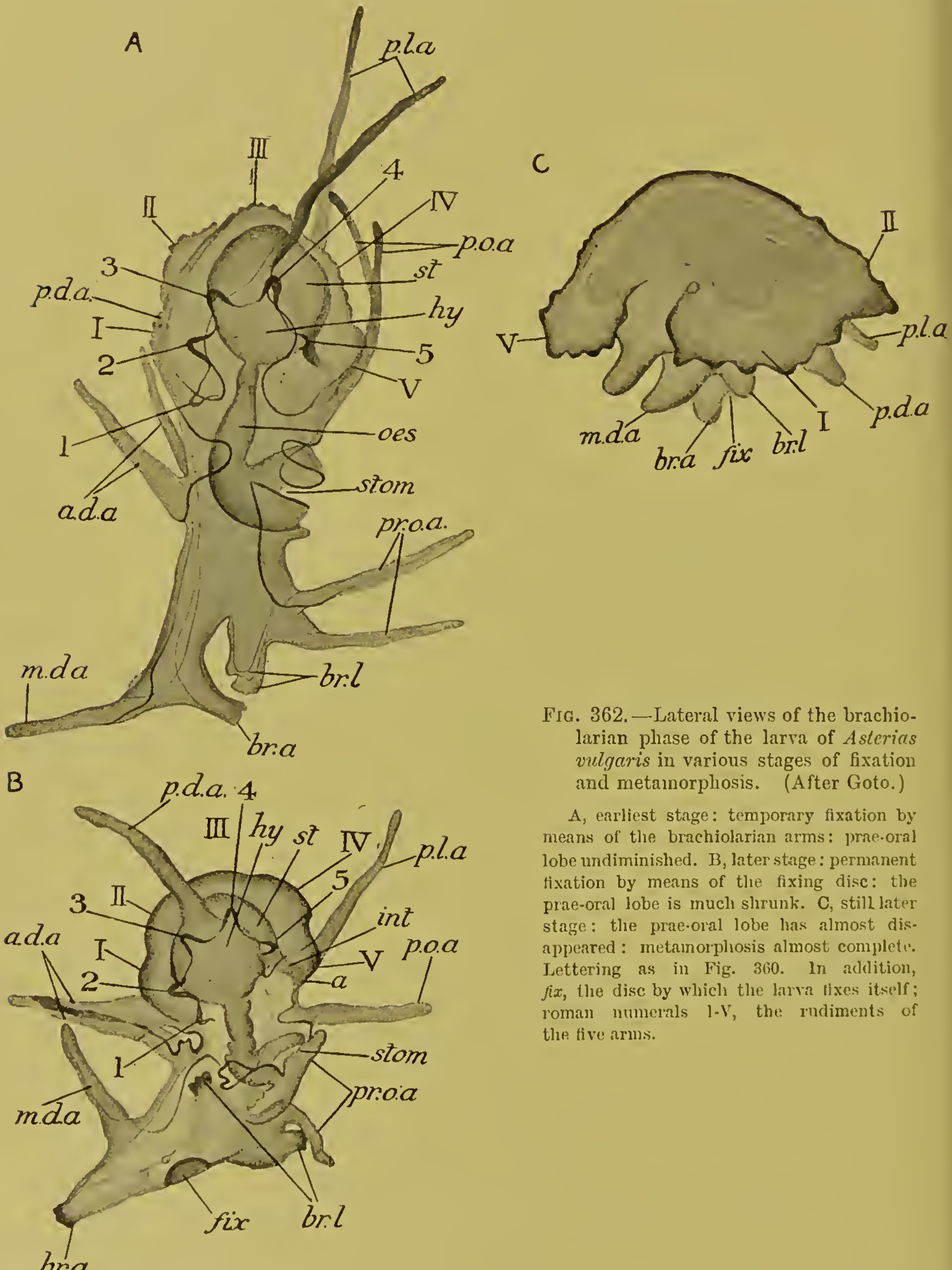

Fig. 362.—Lateral views of the brachiolarian phase of the larva of *Asterias vulgaris* in various stages of fixation and metamorphosis. (After Goto.)

A, earliest stage: temporary fixation by means of the brachiolarian arms: prae-oral lobe undiminished. B, later stage: permanent fixation by means of the fixing disc: the prae-oral lobe is much shrunk. C, still later stage: the prae-oral lobe has almost disappeared: metamorphosis almost complete. Lettering as in Fig. 360. In addition, *fix*, the disc by which the larva fixes itself; roman numerals I-V, the rudiments of the five arms.

eventually come to occupy a position at the posterior pole of the larva. At the same time other changes are occurring. The hydrocoele becomes more and more grooved off from the anterior coelom. The stone-canal is changed from a groove into a tube by the meeting of its edges. As the hydrocoele becomes grooved off it exhibits a dorsal horn and a ventral horn, and a central piece where it remains for a time in connection with the anterior coelom. The ventral horn extends round underneath the larva to the right side, in fact it grows parallel with the left posterior coelom.

Thus the metamorphosis of the larva may be roughly summed up as consisting in the preponderant growth of the organs of the left side as compared with their antimeres on the right side (right hydrocoele and right posterior coelom), together with the gradual atrophy of the prae-oral portion of the larva which forms the stalk. The lobes of the hydrocoele are the rudiments of the **radial water-vascular canals** of the adult star-fish, and the completed arm consists of the process of the aboral disc, into which an outgrowth from the left posterior coelom extends, and to which the process of the hydrocoele becomes applied. There is little doubt that the process of the aboral disc, with its contained coelom, is to be regarded as an outgrowth of the body, secondarily developed, in order to give support to that long hydrocoele lobe, or radial canal, which was originally a free tentacle. Each primary lobe of the hydrocoele develops lateral lobes in pairs, as branches; of these two pairs are formed before metamorphosis is complete. These are the rudiments of the **paired tube feet.**

The **adult stomach** appears, we have seen, as an outgrowth from the larval stomach on the left side, in the region where the peri-oral coelom has made its appearance as an outgrowth from the left posterior coelom. As the new stomach grows out the peri-oral coelom extends round it; whilst outside it the left posterior coelom, whose dorsal and ventral horns meet and fuse with one another, forms an outer ring. At the same time short pouches grow out from the larval stomach into the developing arms. These pouches are the rudiments of the **pyloric caeca** of the adult, and the larval stomach becomes the **pyloric sac**, whilst the adult stomach is really the eversible sac which the star-fish wraps round its prey. The **adult mouth** is formed by the fusion of the adult stomach with the ectoderm.

Only when the metamorphosis is nearly complete does the **rectum** make its appearance. It grows out in the mesentery separating the right posterior coelom from the left posterior coelom, in the same mesentery, that is to say, in which the larval intestine was situated, and, as mentioned above, Gemmill has shown that it is formed from the stump of the larval intestine. The **adult anus** appears later still; it is eventually perforated in the right dorsal inter-radius, using the word "right" in reference to the sagittal plane of the larva.

The shrinkage of the prae-oral lobe is largely due to the change in form of the ectoderm cells covering it—they change from a flat to a columnar form. The ectoderm covering the brachiolar arms is involuted into pockets, and these involuted portions are attacked and devoured by phagocytes. In *Asterina* all the ectoderm of the prae-oral lobe is disposed of in this way; but in *Asterias*, as the prae-oral lobe shrinks in size, a good deal of the ectoderm which originally covered it is drawn into the covering of the oral disc of the star-fish. As the shrinkage of the prae-oral lobe goes on, the sinuosities of the ciliated band, the "arms" of the larva, become straightened out and thus obliterated. Finally, all that is left of the prae-oral lobe is a small button projecting from the oral surface of the star-fish (Fig. 362, C), but when the star-fish begins its free life, as it wrenches itself free, the neck of this button is pulled out into a long filamentous stalk which eventually breaks through and sets the star-fish free.

DEVELOPMENT OF OTHER ASTEROIDEA

We may now glance at the peculiarities of the larvae of other Asteroids whose life-history has been studied. Quite a number of "species" of Bipinnaria are known, but few of them can be assigned to any definite species of Asteroid. The complete life-cycle is only known in the case of *Asterias rubens*, *A. glacialis*, and *A. vulgaris*.

It has been doubted whether all Bipinnaria larvae develop brachiolar arms, *i.e.* pass through a fixed stage. Gemmill has recently shown that the larva of *Porania pulvillus* has such a stage; but extraordinary statements are made about the large Bipinnariae belonging to Asteroids of the family Astropectinidae, in which brachiolar arms have never been observed. M. and C. Delap (1905) state that in these larvae the star-fish rudiment is amputated from the posterior half of the larva, the front half of which goes on living for a long time after. Here is a matter which urgently requires reinvestigation.

We may now turn to the consideration of the development of *Asterina gibbosa*, which has been worked out by us in considerable detail (MacBride, 1896). This development has already been alluded to more than once. Its main peculiarities concern (*a*) the formation of the larval gut and of the coelom, and (*b*) the external appearance of the larva.

With regard to (*a*) the first point, we find that *Asterina gibbosa* has a prolonged embryonic life and only escapes from the egg-membrane on the fourth day, when not only coelom, but also stomodaeum and madreporic pore have been formed. The archenteron is spacious and nearly fills the blastocoele. The coelom arises as an enormous unpaired vesicle, constituting more than half the archenteron, and from this vesicle prolongations, "tongues," extend backwards at the sides of the gut (Fig. 363, B). Then transverse septa appear, which divide off right and left posterior coeloms from an anterior unpaired coelom. These septa are found in the "tongues" of the coelom, so that there is an anterior portion of each tongue which belongs to the anterior coelom. The larval intestine is straight, and both it and the larval anus disappear shortly after the animal enters on its larval existence; that is on the fifth day. The coelom is only separated from the gut after the stomodaeum has broken through.

As for the second point (*b*), the larva has the form of a boot. The sole of the boot is the prae-oral lobe, which is enormous, and the "upper" of the boot is the body. The back of the boot corresponds to the ventral surface of the larva, and here the larval mouth is situated; while the front of the boot is the dorsal surface. The prae-oral lobe is surrounded by a thickened ridge which bears specially long cilia, by the aid of which, and of the cilia of lesser length which cover the ectoderm everywhere, the larva glides about on the bottom. It uses the prae-oral lobe as a sucker, attaching the thickened rim to the substratum on which it is moving, and then retracting the centre. In the centre there appears, on the seventh day, a circular area of thickened glandular epithelium (Fig. 364). This is the fixing disc, and by means of the secretion produced by it the larva effects a permanent fixation to the bottom. Once this has been accomplished the rim is destroyed by the same process as that by which the brachiolar arms are removed in the Bipinnaria larva. The whole prae-oral lobe shrinks, until final atrophy takes place and the larva wrenches itself free and walks away as a little star-fish.

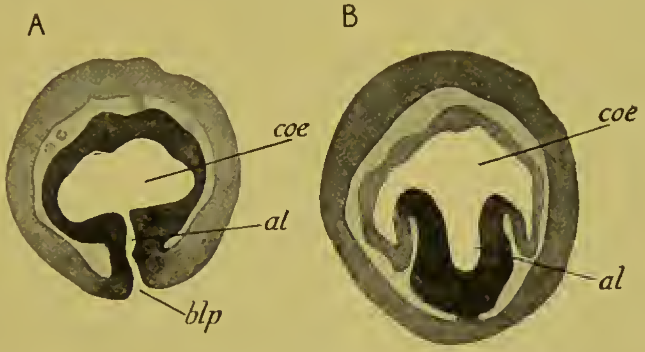

FIG. 363.—Frontal longitudinal sections of two early embryos of *Asterina gibbosa*, in order to show the development of the coelom. (Original.)

A, stage in which the coelom is a spherical vesicle. B, stage in which the coelom is growing back in tongues at the sides of the gut. *al*, rudiment of gut: *blp*, blastopore; *coe*, rudiment of the coelom.

The internal changes which take place during the larval life, and the metamorphoses, are known in detail. Let us go back to the time when the transverse septa are found in the coelom. The septum is formed on the left side before it is formed on the right, and in both cases it begins at the dorsal side and grows down to the ventral surface. On the left side, the septum, after formation, becomes perforated by two holes, a dorsal and a ventral one. In this way a free passage of fluid between anterior and posterior coeloms is allowed; and as the cells of the coelomic wall, as in the Bipinnaria larva, secrete muscular fibrils, and the larva can change its shape very much, this is very necessary. Similar holes are formed in the transverse septa in the Bipinnaria larva, as we have already seen.

The hydrocoele arises as a bulge on the left side of the posterior part of the anterior coelom, whilst the madreporic vesicle is formed as a bud from the posterior end of the anterior coelom, a little to the right of the median line. It becomes hollowed out, and is for a time attached to the wall of the anterior coelom by a string of cells, but this is soon broken and the vesicle detached. A right hydrocoele with five well-developed lobes, or sometimes with only three or only one lobe, is sometimes developed, and often in this case the madreporic vesicle is suppressed, which is the reason why we formerly regarded the two structures as homologous. Even before metamorphosis begins the left posterior coelom is wider than the right, and begins to send out a ventral horn which underlies the right coelom, and the peri-oral coelom originates as a pocket of the left posterior coelom.

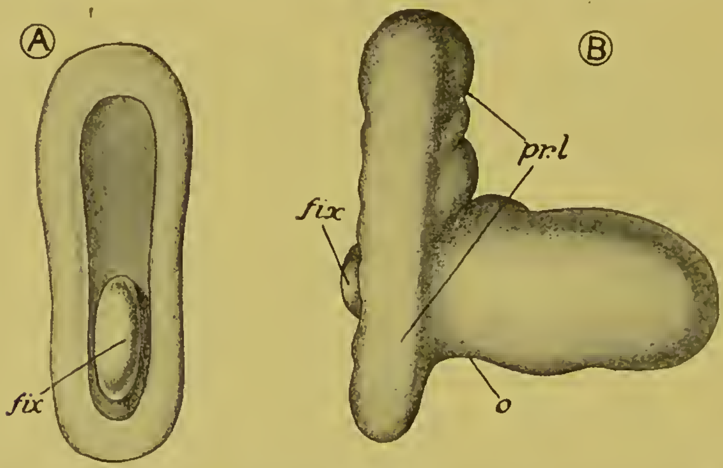

FIG. 364.—Views of a free-swimming larva of *Asterina gibbosa* five or six days old. (After Ludwig.)

A, from in front. B, from the side and above. *fix*, fixing disc. *pr.l*, prae-oral lobe.

In *Asterina gibbosa*, as soon as metamorphosis commences the stone-canal makes its appearance as an open groove on the anterior face of the transverse septum, as in *Asterias*. This groove becomes closed in the middle, but opens at one end into the hydrocoele, and at the other end into the anterior coelom just below the opening of the pore-canal. Then five outgrowths of the coelom shaped like inverted wedges are formed. Of these outgrowths, one arises from the anterior coelom and four from the left posterior coelom (*p.h*, Fig. 366). They project against the ectoderm and alternate with the five lobes of the hydrocoele. These outgrowths are soon cut off from the coelom, and lie between ectoderm and coelomic wall as flattened vesicles. They are the rudiments of the **perihaemal** system of spaces.

The arms grow out as blunt outgrowths from the region of the body occupied by the left posterior coelom, and into each of them an outgrowth from this coelom extends. It is noteworthy that, counting the arms from before backward, No. 1 arm is really situated over No. 2 lobe of the hydrocoele, and eventually fuses with it; and later in the metamorphosis, when the ring-shaped growth of the left hydrocoele and the left posterior coelom is complete, No. 1 hydrocoele lobe comes to lie under No. 5 arm. The neighbouring angles of adjacent perihaemal spaces grow into the arms beneath the hydrocoele lobe, and in this way the two **radial perihaemal canals**, which are found in each adult arm, are formed. The **external perihaemal ring-canal** is formed by the fusion of the main portions of these spaces.

The **internal perihaemal canal** is formed by a circular extension of the hinder part of the anterior coelom which is included within the body of the star-fish when the stalk finally disappears. This portion of the anterior coelom, into which pore-canal and stone-canal open, is known as the **axial sinus** (Fig. 366, a^1c^1). The septum, which divides it from the general body-cavity surrounding the stomach in the adult star-fish, is nothing but the old transverse septum which separated the anterior coelom from the left posterior coelom in the larva; and it follows, therefore, that the general body-cavity of the adult is only the ring-shaped left posterior coelom, which, with Goto, we may term the **hypogastric coelom**. The right posterior coelom of the larva becomes the **epigastric coelom** of the adult.

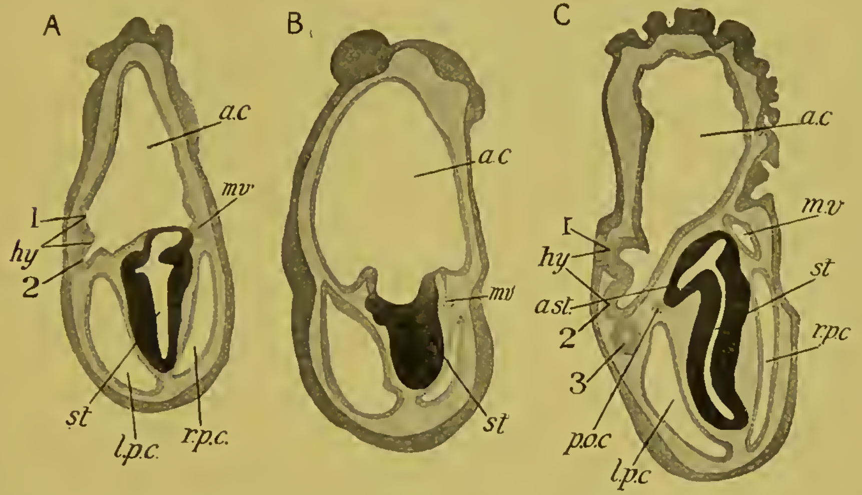

FIG. 365.—Longitudinal frontal sections of larvae of *Asterina gibbosa*, to show the segmentation of the coelom and the origin of the hydrocoele and madreporic vesicles. (Original.)

A, section of larva about five days old. B, section of larva about six days old. C, section of larva about six and a half to seven days old. *ac*, anterior coelom; *a.st*, rudiment of adult stomach; *hy*, rudiment of the hydrocoele; 1, 2, etc., its lobes; *l.p.c*, left posterior coelom; *m.v*, madreporic vesicle; *p.o.c*, rudiment of peri-oral coelom. *r.p.c*, right posterior coelom; *st*, larval stomach.

In *Asterias*, Goto maintains that the perihaemal spaces appear as solid masses of mesenchyme, lying on the ventral surfaces of the arms when metamorphosis is complete, and that these spaces subsequently become hollowed out; but Gemmill has shown that this is an error and that these spaces originate in *Asterias* in the same manner as they arise in *Asterina.*

As metamorphosis proceeds the prae-oral lobe shrinks more and more, and the neck of the lobe becomes constricted. The effect of this is to bring arm No. 5 closer and closer to hydrocoele lobe No. 1 (Fig. 368). At the same time each hydrocoele lobe gives rise to two pairs of lateral branches springing from its base. These are the rudiments of the paired tube feet (Fig. 368, *t.f*), while the tip of the primary lobe forms the azygous tube foot in which the radial canal terminates.

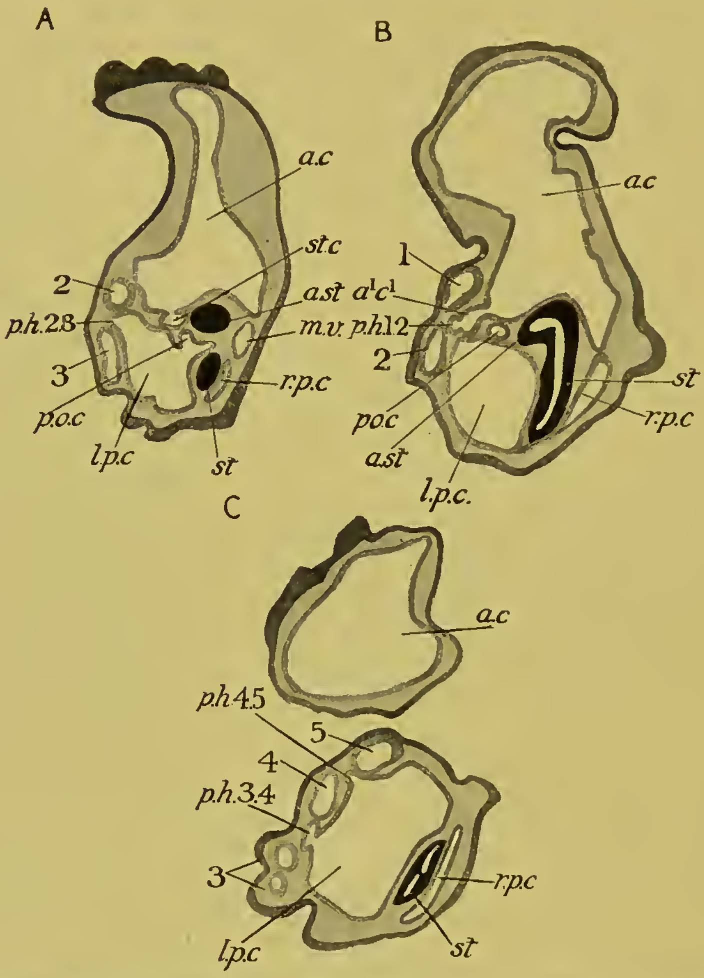

FIG. 366.—Longitudinal frontal sections of larvae of *Asterina gibbosa* seven to eight days old, to show the beginning of the metamorphosis. (Original.)

A, section through a larva in the dorsal region of the body. B, section through a larva near the median region of the body. C, section through a larva in the ventral region of the body. Letters as in previous figure. In addition, *p.h*, rudiments of perihaemal spaces; *p.h.*1.2, rudiment of perihaemal space intervening between lobes 1 and 2 of the hydrocoele; *p.h.*2.3, rudiment of perihaemal space between lobes 2 and 3 of the hydrocoele, and so on; *st.c*, stone-canal (still an open groove).

The larval oesophagus or stomodaeum, as in *Asterias*, becomes disconnected from the larval stomach, shallows out and disappears. The larval stomach, which, as we have seen, forms the adult pyloric sac, begins to give off blunt outgrowths into the cavities of the nascent arms: these are the rudiments of the **pyloric caeca** (Fig. 369). The adult stomach, begun as we have noted as an outgrowth on the left side of the larval stomach, increases in size and comes in contact with the ectoderm at a spot between the dorsal and ventral horns of the left posterior coelom. These horns meet above it, and so the left posterior coelom is converted into a ring. Within this ring lies the ring formed by the hydrocoele, and beneath this the ring formed by the peri-oral coelom. The holes in the septum dividing the anterior coelom from the left posterior coelom become healed up, and with the progressive constriction of the neck of the prae-oral lobe the anterior coelom becomes divided into a transitory portion, situated in the stalk, and a permanent portion, the axial sinus, which is included within the disc of the star-fish. The hydrocoele communicates with the anterior coelom not only through the stone-canal, which has become a closed tube, but through an opening in the neighbourhood of its third lobe which does not become closed until metamorphoses is nearly complete.

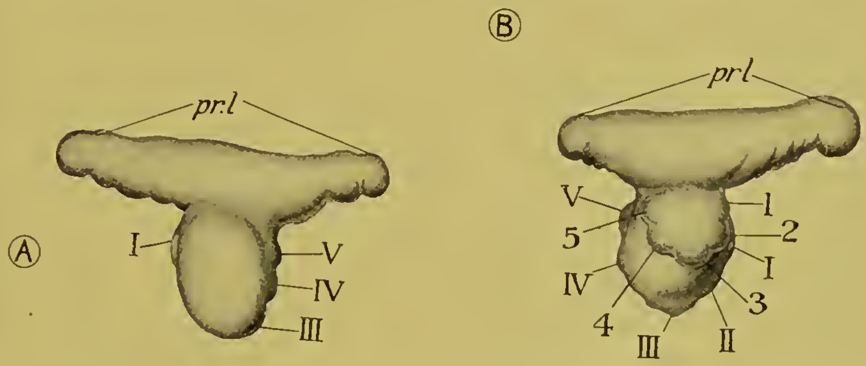

Fig. 367.—Views from the side of a larva of *Asterina gibbosa* seven days old in the initial stages of metamorphosis. (After Ludwig.)

A, from the right side. B, from the left side. 1-5, lobes of hydrocoele. I-V, rudiments of arms; *pr.l*, prae-oral lobe.

The **adult nervous system** of *Asterina* arises as a plexus of ganglion cells and fibres, beneath the ectoderm which overlies the perihaemal spaces and the lobes of the hydrocoele. Goto states that, in *Asterias*, part of this ectoderm is derived from the longitudinal ciliated band of the larva. If this statement could be confirmed it would be a matter of great interest.

Of the development of the adult **calcareous skeleton** of *Asterina* we have a full account from Ludwig (1882), and we have also some information about the origin of the calcareous skeleton in *Asterias rubens* from Bury (1895). The first traces of calcareous plates in

Asterina, and in all other Echinoderms which have been studied, are little triradiate spicules embedded in and produced by the mesenchyme cells intervening between coelomic wall and ectoderm; each arm of the spicule, as it grows, bifurcates, and the forks of adjacent arms join one another, and in this way a mesh is formed. From the junction of the two forks another arm is given off, and, by a repetition of the processes of forking and of union of forks, a network of calcareous meshes is slowly built up.

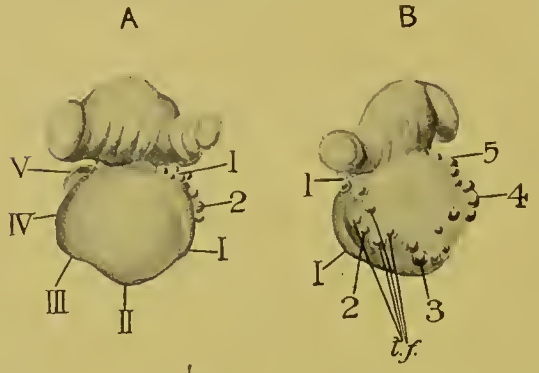

Fig. 368.—Views from the side of a larva of *Asterina gibbosa* eight days old, to show the progress of metamorphosis. (After Ludwig.)

A, from the right side. B, from the left side. Letters as in previous figure. In addition, *t.f*, rudiments of paired tube feet.

The first spicules to appear in *Asterias* and *Asterina* are the rudiments of the **terminal plates** (T, Fig. 370), which overarch and protect the azygous tentacles in which the primary lobes of the hydrocoele, or radial water-vascular canals, terminate. Alternating with these terminal plates arise five **basal plates** (B, Fig. 370), one of which surrounds the madreporic pore and is the rudiment of the **madreporite** of the adult. In the centre of the circle of basals there arises the so-called **dorso-central plate** (*D.C*, Fig. 370). This plate does not lie over the right posterior coelom but rather to one side of it, and the adult anus, which appears at one side of the dorso-central plate, is consequently situated over the mesentery separating the left posterior and right posterior coeloms.

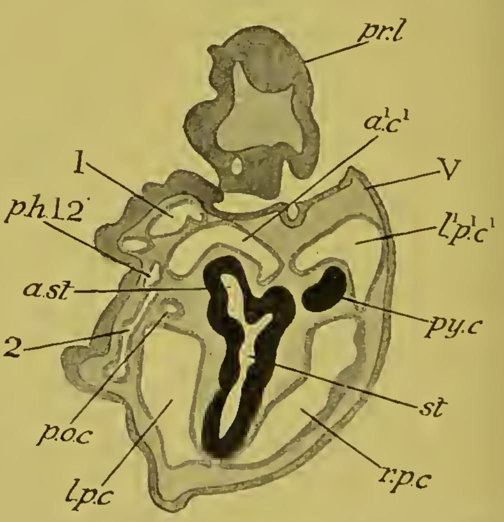

Fig. 369.—Longitudinal frontal section through a larva of *Asterina gibbosa* about the same age as those shown in previous figure. (Original.)

Letters as in Figs. 365 and 366. In addition, a^1c^1, rudiment of axial sinus; *py.c*, rudiment of pyloric caecum.

The rectum, which is formed as an outgrowth from the larval stomach, lies in this mesentery. On the ventral side of the disc there appear pairs of spicules alternating with the rudiments of tube feet. These spicules are the rudiments of the **ambulacral plates**. The muscles connecting these plates with one another, by means of which the arm can be bent and the ambulacral groove closed, are derived from the cells forming the walls of the perihaemal canals.

As metamorphosis approaches completion, the septum dividing the peri-oral coelom from the encircling left posterior coelom is largely absorbed, and the two cavities coalesce; as remnants of this septum there remain ten bands, two in each arm, which constitute the **retractor muscles** of the adult stomach. The adult mouth is formed by the fusion of the wall of this stomach with the ectoderm; there is no adult stomodaeum. When the stalk has been almost absorbed the little star-fish wrenches itself loose from the substratum by pulling with its tube feet, and it walks away.

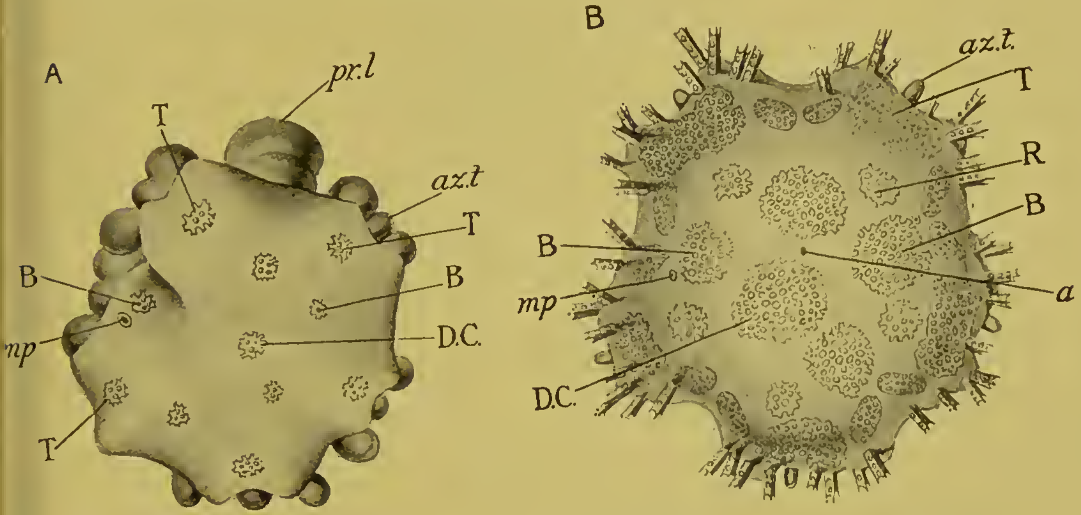

FIG. 370.—Dorsal (*i.e.* aboral) views of two young specimens of *Asterina gibbosa* shortly after the metamorphosis. (After Ludwig.)

A, young star-fish ten days old. B, young star-fish sixteen days old. *a*, adult anus; *az.t*, azygous tentacle of water-vascular system; *B*, basal plate; *D.C*, dorso-central plate; *R*, radial plate; *T*, terminal plate. In the specimen shown in figure B, only four basals are developed.

The post-larval development has been followed in *Asterina*. The main points which have been determined concern the further development of the skeleton and the development of the genital system. We shall deal with the development of the skeleton first. As the arms grow in length new tube feet are added in pairs, the first formed tube feet remaining at the base of the arm, and, alternating with the new tube feet, new ambulacral ossicles are added. At the same time the terminal plates are carried out to the tips of the arms, and new plates are intercalated between them and the central plate. The most important of these, and the first to appear, are the **radials** situated at the bases of the arms. The names basals, dorso-central, and radials, it may be remarked, have been bestowed on these plates from a suggested homology with the plates which make up the skeleton of a Crinoid, the value of which will be examined when we consider the development of a Crinoid.

The origin of the **genital organs** is very peculiar. About the time that the metamorphosis is completed a peculiar fold appears in the wall of the axial sinus which abuts against the left posterior, or, as we may now term it, the **hypogastric coelom**. This fold is the rudiment of the strand called **axial organ, dorsal organ**, or **genital stolon**, and formerly regarded as a heart (*d.o*, Fig. 371, A). At the dorsal edge of the hypogastric coelom, where the remains of the mesentery which separated it from the epigastric coelom are still to be seen, an involution of the coelomic epithelium takes place which

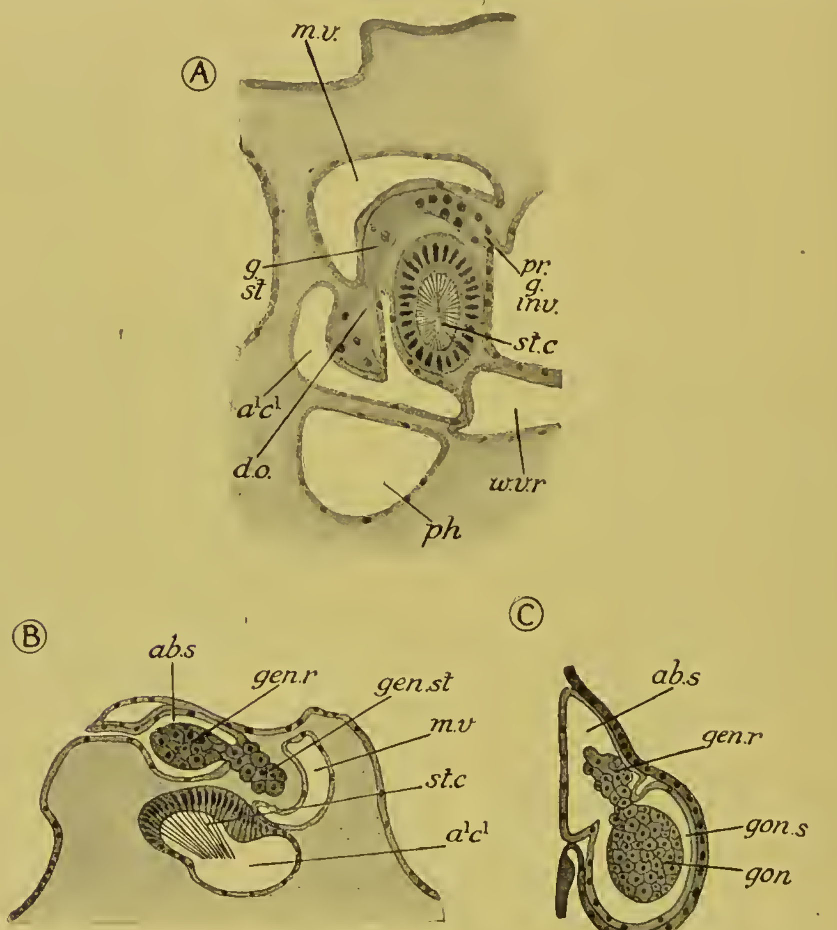

FIG. 371.—Three figures to illustrate the development of the genital stolon, genital rachis, and gonad in *Asterina gibbosa*. (Original.)

A, vertical section of the disc of a specimen that has just metamorphosed. Disc about ·75 mm. in diameter. B, horizontal section through one of the inter-radial septa of a specimen about 1·2 mm. across the disc. C, Section through the incipient gonad of a specimen about 6 mm. across the disc. *ab.s*, aboral sinus; a^1c^1, axial sinus; *d.o*, dorsal organ; *gen.r*, genital rachis; *gen.st*, genital stolon; *gon*, gonad; *gon.s*, gonadial sinus; *m.v*, madreporic vesicle; *ph*, perihaemal space; *pr.g.inv*, primitive germinal involution; *st.c*, stone-canal.

projects into this fold. The involution, at first hollow, soon becomes solid and is termed the **primary germinal involution** (*p.g.inv*, Fig. 371, A). The solid bud thus produced proliferates downwards into the axial organ and forms the peculiar cells characteristic of this structure, but it also proliferates laterally, to the right and to the left, and forms a cord of primitive germ cells known as the **genital rachis** (*g.r*, Fig. 371, B), which grows as a freely projecting rod right round the disc of the star-fish until the two ends meet and a complete circle is formed. The rod is, of course, covered with a thin layer of peritoneum which is reflected over it; it is supported in a sling of peritoneum which grows out parallel with the rod and underneath it, as a flap, which fuses with the body wall on both sides and encloses a space called the **aboral sinus** (*ab.s*, Fig. 371, B).

At each side of the base of each arm a branch is given off from the rachis enclosed in a branch of the aboral sinus. This branch extends downwards and then along the arm, where it enlarges and forms a bunch of diverging branches which constitute the **genital organ**. Round each branch, of course, is a branch of the aboral sinus, but this branch becomes cut off from the main part of the sinus by the ingrowth of a septum (*gon*, Fig. 371, C). The **genital duct** is formed as a solid outgrowth from the base of the genital organ, where it joins the rachis. This outgrowth fuses with the ectoderm and then becomes hollowed out. The process by which a genital gland discharges itself bears a considerable resemblance to the way in which an abscess finds its way to the surface.

Both *Solaster* and *Cribrella* agree, as far as the general form of their larvae is concerned, with *Asterina*, though differing in details of shape and size of the prae-oral lobe, and they have a quite similar metamorphosis. But they differ in one most important respect, they never develop a larval mouth, and the larval gut remains in a most rudimentary condition, being merely a collar of more columnar cells round the middle portion of the archenteron. The hinder portion of the archenteron gives rise to the left posterior coelom, whilst the front portion gives rise, not only to the single anterior coelom, but also to the right posterior coelom. From the anterior coelom are also given off the hydrocoele and the madreporic vesicle.

The credit of elucidating this extraordinary form of development belongs to Masterman (1902), whose observations on *Cribrella* have been confirmed in almost every point by those of Gemmill on *Solaster* (1912). Masterman (1902), viewing this development as primitive, regards the right posterior coelom as the antimere of the left hydrocoele, and the left posterior coelom as a median structure containing right and left elements. He terms the madreporic vesicle the **central coelom.** According to Gemmill, in *Solaster* the madreporic vesicle arises to the right of the middle line, as in *Asterina*.

There are obvious objections to regarding the shortened development of an Asteroid, with a yolky egg, as capable of throwing light on the primitive condition of the development of Asteroidea. Gemmill's researches (1912) on *Solaster* allow of another interpretation of the type of development. In *Solaster* the posterior coelom is not symmetrical, but inclined to the left, and the area which eventually forms the gut does not extend evenly all round the archenteron. We might therefore derive the condition of affairs in the larva of *Solaster* from the condition of things in the larva of *Asterina*, by imagining that the preponderant growth of the organs of the left side has been pushed so far back into the embryonic period, that the gut-rudiment is swung out of the longitudinal into a transverse position, and so the open end, from which the coelom is cut off, is directed to the left instead of anteriorly.

Solaster is further remarkable for the fact that, although it is a star-fish with many rays, the left hydrocoele has at first only five lobes which all develop simultaneously, and additional lobes are developed much later; so that in one and the same specimen, when the more dorsal lobes of the hydrocoele have already developed lateral tube feet, and the perihaemal spaces in connection therewith have been completely separated from the coelom, the more ventral lobes will still be quite undivided, and the adjacent perihaemal spaces will have the form of shallow evaginations of the coelom. This circumstance seems to indicate that the number five, so characteristic of the rays in all Echinodermata, was characteristic also of the ancestral Echinoderm, and that where many rays are found this is not a survival of a primitive state of affairs, but is a secondary modification.

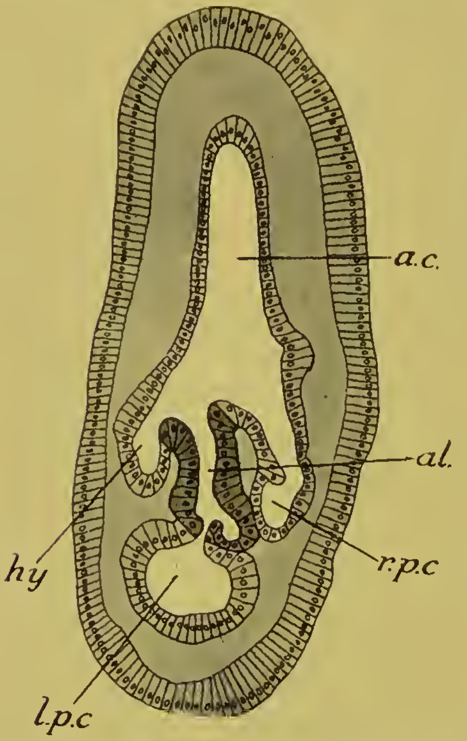

Fig. 372.—Longitudinal frontal section of the larva of *Solaster endeca*. (After Gemmill.)

a.c, anterior coelom; *al*, alimentary canal; *hy*, hydrocoele; *l.p.c*, left posterior coelom; *r.p.c*, right posterior coelom.

EXPERIMENTAL EMBRYOLOGY OF ASTEROIDEA

A great many experiments have been performed on the eggs of Asteroidea, but in the case of many of these quite the same results have been obtained with the eggs of Echinoidea, which have been classic subjects of experimentation since the study of Experimental Zoology started. So far as space will allow they will be mentioned when dealing with the Echinoidea, but there are some which are, so to speak, peculiar to the Asteroidea.

It has been already mentioned that the fully developed gastrula of *Asterias* is a long, sausage-shaped structure, and that the archenteron only reaches half-way through it (Fig. 356). Driesch (1895) made a thick culture of these gastrulae, accumulating hundreds in a very small quantity of water, and snipped at random in this water with a fine pair of scissors about 200 times. In this way he succeeded in cutting a number of the gastrulae in pieces. Sometimes he found that they were cut longitudinally and sometimes transversely. In both cases, if the fragment included both endoderm and ectoderm, it healed up by the approximation of its edges and formed a miniature gastrula, which then developed further into a small but perfect Bipinnaria. If, however, the gastrula was transversely bisected after the thin-walled vesicle at the apex of the archenteron had made its appearance, so that this vesicle was removed, then the truncated larva healed up and went on developing, but it never formed a new coelomic vesicle, although it takes on externally the form of a Bipinnaria. The appearance of this vesicle, therefore, according to Driesch, "negatively determines," *i.e.* limits the potency, that is, the power of development possessed by the archenteric wall. Before that vesicle appears a small fragment of this wall will grow and rearrange its cells so as to form the three segments of the asteroid gut, viz. oesophagus, stomach, and intestine, and in addition the terminal vesicle; but, when this has been once formed, then, even a larger fragment of the archenteron is incapable of moulding itself into more than oesophagus, stomach, and intestine.

Driesch regards this limitation of power as due to a progressive "stiffening" of the protoplasm, which renders it less and less amenable to the regulating influence of the "**entelechy,**" or indwelling power, which, according to the vitalistic principle held by Driesch, *knows and wills what it wants to do with the material at its disposal.* A more humble explanation, and one more in accord with what we know of other eggs, is that there exists a definite **coelom-forming substance** which is at first diffused through all the cells of the gut wall, but which becomes, as development proceeds, definitely localized in one spot; and if that portion of the gut be removed, the remainder has no material which will allow of the development of the coelom.

Herbst (1896) showed that if a solution of 3·7 per cent of sulphocyanide of potassium be made, and then three parts of this solution be added to 100 parts of sea-water, the eggs of *Asterias* will develop in this medium and will live on for four weeks, but that they do not get beyond the blastula stage. The beginning of an archenteron may be formed, but it degenerates into a granular mass of cells and is absorbed.

These persistent blastulae, though possessing the clear transparence which indicates health, differ from the normal blastulae in possessing abundance of mesenchyme in the interior. The normal blastula of *Asterias* does not develop mesenchyme cells, these are only formed after the gastrula stage has been reached. In these abnormal blastulae the mesenchyme cells are given off from the vegetative pole, while from this same pole mesenchyme is given off in the normal blastula of *Echinus* before an archenteron has been formed.

Herbst concludes that the action of the sulphocyanide has changed the development of the blastula from the Asteroid to the Echinoid type. This may be admitted, but the question arises how this comes about. The vegetative pole of the egg is the region which, in the Asteroid, is invaginated to form the archenteron, and from the apex of the archenteron the mesenchyme is given off; so that the mesenchyme originates from the same region in the normal and in the abnormal larva. Further, there is often, as we have seen in the abnormal larva, an attempt to form an archenteron which is, however, abortive.

We may draw the conclusion, therefore, that the normal development consists of distinct processes which are to a large extent independent of one another. On these processes the drug acts in a selective way; it inhibits the process of forming an archenteron, but it permits the formation of mesenchyme. We might go on to suggest that the early development of both Asteroidea and Echinoidea is made up of the same processes, but that in the case of the Echinoidea the mesenchyme development is hurried on. In this case it is not impossible that the hurrying on of the mesenchyme formation in the blastula of Echinoidea is due to the formation of some substance which acts in a similar manner to the sulphocyanide, and that, therefore, one main difference between the eggs of Asteroidea and Echinoidea is the presence of this substance in the latter type of eggs, and its absence in the former.

OPHIUROIDEA

Leaving now the Asteroidea, let us turn to the consideration of the group most nearly allied to them, the Ophiuroidea. The development of three species of Ophiuroids is known, viz. *Ophiothrix fragilis*, *Ophiura brevispina*, and *Amphiura squamata*. Of these only the first possesses a long larval development comparable with that of *Asterias*, and we shall therefore select it as type for special description. Its development has been worked out by us (1907).

Ophiura brevispina has a yolky egg and a much modified larva, which takes no nourishment and creeps slowly about on the bottom, and therefore exhibits a development in some respects parallel to that of *Cribrella* and *Solaster*. *Amphiura squamata* has a development which is passed entirely within a pocket in the maternal body —**the genital bursa**—which acts as a womb, from which the embryo emerges as a star-fish similar to the adult in all important respects.

OPHIOTHRIX FRAGILIS

Methods of preservation and staining which are applicable to the study of *Asterias* are equally applicable in the case of *Ophiothrix*, and it will therefore be unnecessary to repeat them here. One interesting point, however, remains to be noticed. The normal development of the eggs of *Ophiothrix fragilis* was only obtained in the case where these eggs were spawned naturally; this was accomplished by placing the males and females, which are easily recognizable by the colour of the gonad shining through the skin, in pairs, in separate vessels of clean sea-water. The females have a colour which varies from light red to dark-brownish red, the males, on the contrary, are of a light yellow or buff colour. The eggs are very small, not more than ·1 mm. in diameter, and are rendered quite opaque by the dense accumulation of red yolk grains.

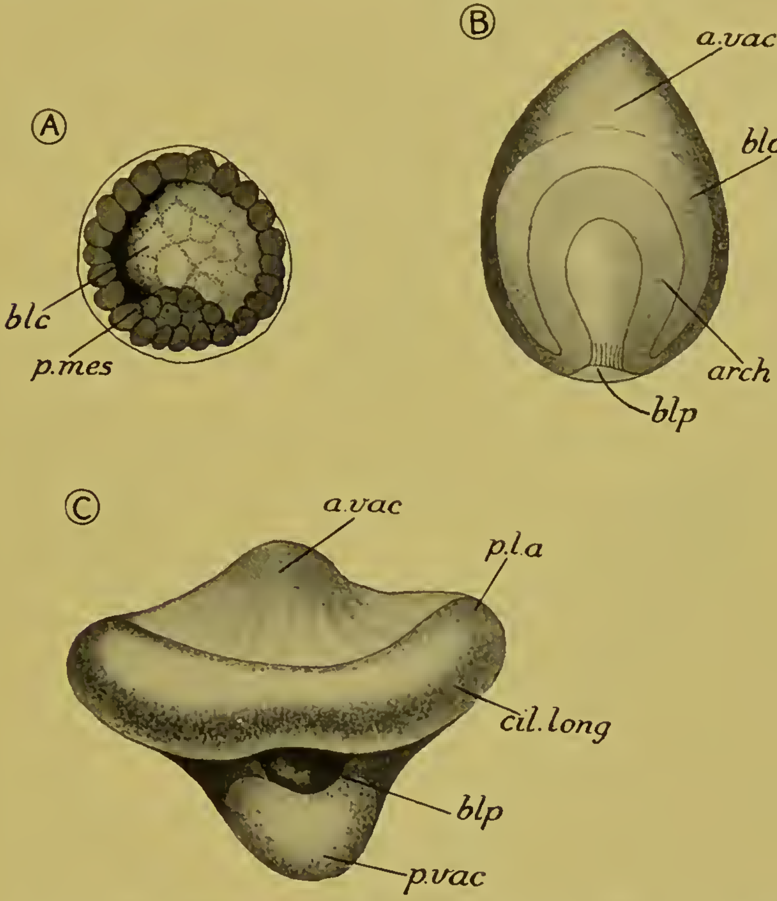

FIG. 373.—Early stages in the development of *Ophiothrix fragilis*. (Original.)

A, early blastula, seven hours old. B, gastrula, twenty-one hours old. C, Larva, thirty hours old, viewed from the ventral side. *arch*, wall of archenteron; *a.vac*, anterior vacuolated crest; *blc*, blastocoele; *blp*, blastopore; *p.mes*, primary mesenchyme; *p.vac*, posterior vacuolated crest.

Segmentation takes place with great rapidity, and in four or five hours a small spherical **blastula**, consisting of relatively few cells, is formed. Then **primary mesenchyme** (*p.mes*, Fig. 373, A) becomes budded off from one pole, and occupies a large portion of the **blastocoele**. The side of the blastula from which this is budded off is of course the vegetative pole. The opposite pole of the blastula develops into a great conical protuberance, which we may term the **anterior vacuolated crest**. This arises (*a.vac*, Fig. 373, B) as a consequence of the growth in height of the cells which form the blastula wall in this region. They become changed into elongated pillars, and develop clear vacuoles in their interior (*a.vac*, Fig. 374, A),

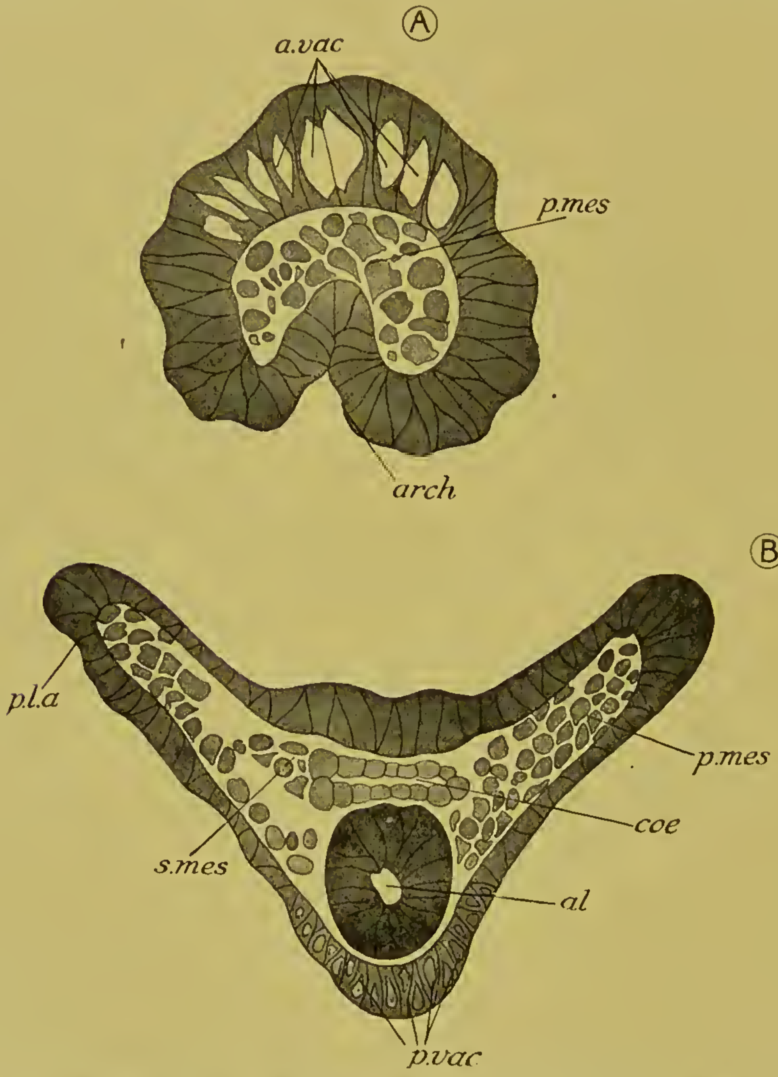

FIG. 374.—Longitudinal frontal sections of early larvae of *Ophiothrix fragilis*. (Original.) *al*, alimentary canal; *arch*, archenteron; *a.vac*, anterior vacuolated crest; *coe*, rudiment of coelom; *p.l.a*, postero-lateral arm; *p.mes*, primary mesenchyme; *p.vac*, posterior vacuolated crest; *s.mes*, secondary mesenchyme.

and the vacuoles almost certainly consist of some material which is lighter than water. Thus, while the general shape of the embryo becomes conical, the outline of its cavity, the blastocoele, remains spherical.

The blastula now acquires cilia, bursts the egg-membrane, and enters on a free swimming existence. Before eighteen hours have elapsed the invagination to form the archenteron has begun, and the blastula has become a **gastrula.** **Secondary mesenchyme** is given off from the apex of the archenteron as this is being formed. When the archenteron is fully formed it swells out at its free end into a thin-walled vesicle, which is the rudiment of the coelom (*coe*, Fig. 374, B). In *Asterias*, as we have seen, there is also formed a single vesicle at the apex of the archenteron, but, according to the current account, the coelom originates as two sacs which are cut off separately from the archenteron.

In *Ophiothrix*, however, when this vesicle becomes cut off from the archenteron, it persists for a brief time as a single vesicle; then it becomes divided into right and left halves, which lie at the sides of the gut and form the **right and left coelomic** sacs. At the same time two lateral outgrowths of the body appear in the larva, which has up till now had a conical shape (*p.l.a*, Fig. 373 C); these are the rudiments of the **postero-lateral arms,** and into them passes nearly all the primary mesenchyme. The cilia, which until now have covered the whole body, become restricted to a circular band of thickened ectoderm which passes over these arms. This band is, of course, homologous with the **longitudinal ciliated band** of the Bipinnaria larva. In the mesenchyme at the base of each rudimentary arm there appears a little **tri-radiate spicule** of calcium carbonate. Of its three rays one extends downwards towards the posterior pole of the larva, this is termed the **body-rod**; one extends outwards into the arm and is termed the **postero-lateral rod**; and the third extends in an anterior direction and is termed the **antero-lateral rod** (*a.l.a*, Fig. 375, A).

The **stomodaeum** makes its appearance just behind the vacuolated crest on what will prove to be the ventral side of the larva. As it is being formed, the gut, from which the coelom has detached itself, becomes marked out into three regions, viz. **oesophagus, stomach,** and **intestine,** by the appearance of two constrictions. The stomodaeum joins the oesophagus, and when this has been accomplished the alimentary canal is complete. The opening of invagination, or blastopore, persists as the **larval anus.** An **adoral band of cilia** is formed just in the same way as it is formed in the Bipinnaria, and doubtless fulfils the same function. At the same time the two larval arms grow rapidly in length, and the portions of the ciliated band which pass over them are pulled out into long loops. The vacuolated crest diminishes in size and soon disappears completely, and the whole organism takes on in consequence the form of a V. The growth of the larval arms appears to be due to the growth of the calcareous rods contained in them, not because the growth of the arm is due to a passive stretching of the ectoderm, but rather because the pressure of the growing tip of the rod stimulates the ectoderm to increased growth. If the larva be exposed to unfavourable conditions, such as lack of oxygen, etc., the ectoderm shrinks, and the pointed tip of the calcareous rod projects as a naked spine, so that if there were any stretching of the ectoderm the spine would immediately pierce it.

As the anterior vacuolated crest disappears, another, consisting of a precisely similar ridge of ectoderm, makes its appearance at the posterior pole of the larva, and this second crest persists through the entire period of larval development. This we may term the **posterior vacuolated crest.** The purpose of these crests seems to be to diminish the specific gravity of the larva, and so balance the increase in weight due to the development of calcareous matter. The posterior rod of the calcareous star on each side meets its fellow just above this posterior crest. The lower ends of these "body-rods" bifurcate, and, by the meeting of the forks

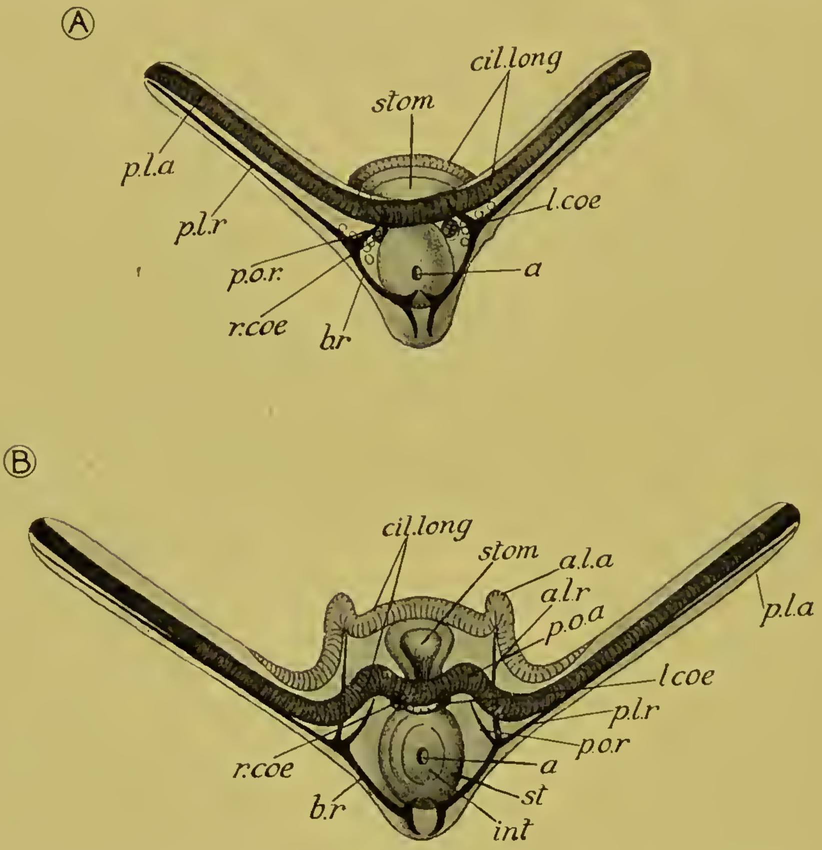

FIG. 375.—Ventral views of young larvae of *Ophiothrix fragilis*. (Original.)

A, larva, two and a quarter days old. B, larva, three days old. *a*, anus; *a.l.a*, antero-lateral arm; *a.l.r*, antero-lateral rod; *b.r*, body rod; *cil.long*, longitudinal ciliated band; *int*, intestine; *l.coe*, left coelomic sac; *p.l.a*, postero-lateral arm; *p.l.r*, postero-lateral rod; *p.o.a*, post-oral arm; *p.o.r*, post-oral rod; *r.coe*, right coelomic sac; *st*, stomach; *stom*, stomodaeum.

a quadrangular calcareous frame is made. When the posterior vacuolated crest is formed the anus becomes displaced on to the ventral surface of the larva, and the whole alimentary canal becomes curved so as to be concave ventrally as in the Bipinnaria larva. The left coelomic sac sends up a vertical outgrowth towards the dorsal surface which meets the ectoderm. At this point the ectoderm becomes vacuolated and then perforated, and so the **primary madreporic pore** is formed. The outgrowth from the left coelomic sac forms the **pore-canal.**

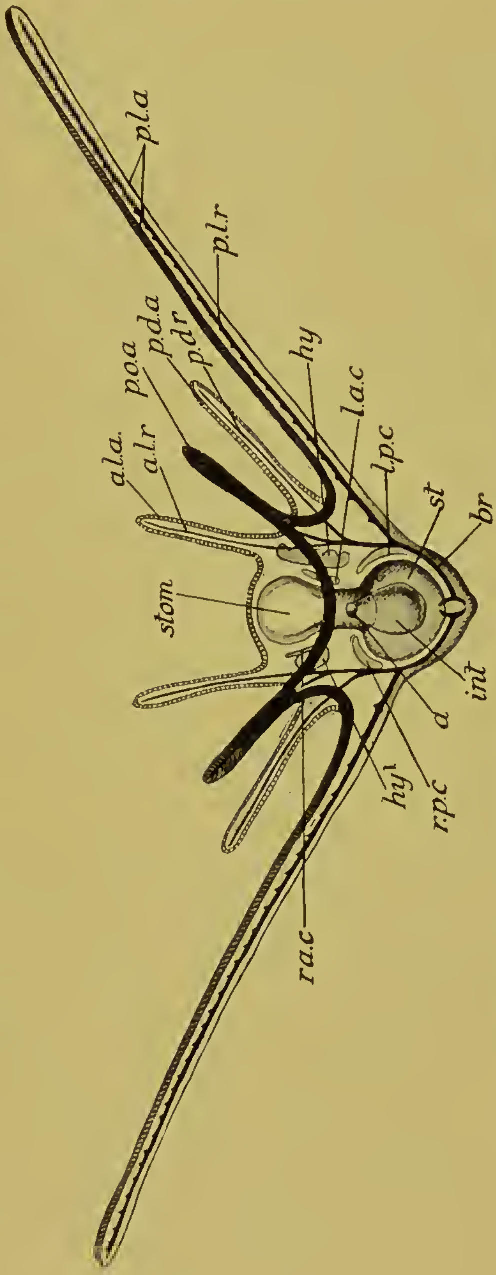

Fig. 376.—Ventral view of fully developed larva of *Ophiothrix fragilis*, sixteen days old. (Original.)

Letters as in preceding figure. In addition, *hy*, hydrocoele; *hy*¹, rudimentary right hydrocoele; *l.a.c*, left anterior coelom; *l.p.c*, left posterior coelom; *p.d.a*, postero-dorsal arm; *p.d.r*, postero-dorsal rod; *r.a.c*, right anterior coelom; *r.p.c*, right posterior coelom.

Whilst these changes are going on another pair of larval arms make their appearance. These are situated at the sides of the mouth, just where the lateral portion of the ciliated band passes into the anterior cross-bar. They owe their origin to the stimulus provided by the growth of the antero-lateral rod and are termed the **antero-lateral arms.** There are no appendages in the Bipinnaria which exactly correspond to these in position.

The larva, not of *Ophiothrix fragilis*, but of the genus *Ophiura*, in the four-armed condition, was seen by Johannes Müller (1845). He thought it a new form of adult marine animal, and named it *Pluteus paradoxus*, from the resemblance it presented to a painter's easel (*pluteus*) on four legs, when turned upside down. Müller, as a matter of fact, orientated the larva wrongly. A year later, however (1846), he bestowed the same name on the similar larva of an Echinoid, which, though generally homologous with the larva of an Ophiuroid, differs from it in most important points of detail. The confusion of having the term "**Pluteus**" applied indifferently to both forms of larvae, led Mortensen (1898) to propose that the single term Pluteus should be replaced by the terms **Ophiopluteus** and **Echinopluteus,** as applied to Ophiuroid and Echinoid larvae respectively, and this excellent practice will be followed here.

When the larva has become endowed with a complete alimentary canal it is able to feed, and it grows rapidly in size. The four arms increase in length, but the first-formed pair, the postero-lateral, grow very much more quickly than the others. The coelomic sacs grow in length and extend backwards along the sides of the oesophagus, and beyond the junction of this with the stomach to the sides of the latter.

When the larva is about a week old important changes occur. Two additional pairs of arms are developed; of these one pair are situated at the sides of the oesophageal region; they correspond roughly in position to the appendages of the Bipinnaria known as **postero-dorsal,** and receive the same name. The other pair arise in front of the anus, from the posterior cross-bar of the ciliated band, and are termed **post-oral,** since they correspond to the similarly named appendages of the Bipinnaria. Each new arm is supported by a calcareous rod which is developed as a new branch from one of the two original calcareous stars. The rods supporting the post-oral arms take their origin from the centre of the star, on each side, which thus becomes quadri-radiate, but those supporting the postero-dorsal rods spring, as branches, from the rods which support the antero-lateral arms.

Whilst these changes have been occurring the coelom has been undergoing further development. First on the left side and then on the right, the coelomic sac becomes transversely segmented into anterior and posterior divisions. The posterior divisions apply themselves to the sides of the larval stomach, which has now become globular; thus these posterior divisions are somewhat widely separated from the anterior divisions, which lie close to the larval oesophagus, just over the region occupied by the posterior part of the adoral ciliated band.

It is obvious that the anterior part of each coelomic sac corresponds to the **anterior coelom** of the Bipinnaria, and the posterior portion to the **posterior coelom** of that larva. From the inner walls of the anterior coelom on both sides are developed muscular fibres which form a series of circular muscles surrounding the oesophagus, and by which its peristaltic movements are carried out. All the arms grow rapidly in length, but the postero-lateral outgrow all the rest and the V form of the larva is maintained.

From the posterior ends of the right and left anterior coeloms a thick-walled vesicle is nipped off. These are the rudiments of the

hydrocoele on the left and of a vestigial **right hydrocoele** on the right; they are at first quite similar to one another. Nevertheless the left side of the larva is clearly distinguishable from the right because the pore-canal opens on the left side, and the portion of the anterior coelom into which it opens becomes distended so as to form an **ampulla.** This ampulla is connected with the rudiment of the left hydrocoele by a narrow neck which later forms the **stone-canal.** On the right side the right hydrocoele remains close to the right anterior coelom. Further, while the lumen of the hydrocoele becomes enlarged, and the cells forming its wall arrange themselves in a single layer of regular columnar epithelium, the lumen of the right hydrocoele remains small and the cells remain rounded and of irregular arrangement.

BIPINNARIA AND OPHIOPLUTEUS LARVAE COMPARED

The larva has now reached the height of its development, and from this period may be dated the beginning of its metamorphosis into the adult Ophiuroid. Before considering this it will be instructive to compare the Ophiopluteus larva, point for point, with the Bipinnaria at a similar stage of development, viz. just before the appearance of the Brachiolarian arms.

The two larvae agree in the following points:—

(*a*) Both have a similar alimentary canal, consisting of a wide shovel-shaped stomodaeum, cylindrical oesophagus (along the sides of which and of the stomodaeum runs the V-shaped adoral ciliated band), a globular stomach, and cylindrical intestine ending in ventral arms. In both the alimentary canal is bent in a curve which is concave ventrally.

(*b*) Both have a similar organ of locomotion, consisting of a longitudinal ciliated band composed of two longitudinal sides and anterior and posterior cross bars. In both this band is produced into long arms or processes which extend its surface.

(*c*) In both the space between gut and skin is filled by a watery gelatinous connective tissue.

(*d*) In both there are a pair of coelomic sacs which become divided into anterior and posterior moieties; and in both the rudiment of the water-vascular system arises from the posterior end of the left anterior sac, and into this sac the pore-canal opens.

On the other hand the Bipinnaria and the Ophiopluteus larva differ in the following points:—

(*a*) The Bipinnaria has a long prae-oral lobe, and the loop of the longitudinal ciliated band, which is bent along the ventral side of this lobe, becomes separated from the rest as a distinct prae-oral band of cilia. The prae-oral lobe is obsolete in the Ophiopluteus, and the longitudinal ciliated band remains undivided.

(*b*) The processes of the ciliated band remain soft in the Bipinnaria, and are usually short in comparison with the size of the body; they become, however, much longer in other forms of Bipinnaria than the one described. In the Ophiopluteus they are very long in comparison with the size of the body, especially the postero-lateral ones, and are supported by calcareous rods; but in other forms of Ophiopluteus larva they are not so long as in the one selected as type.

(*c*) The two anterior coeloms extend into the prae-oral lobe in the Bipinnaria, and fuse there into one anterior cavity; also the septa dividing anterior from posterior coeloms are formed late, and that on the left side undergoes secondary perforations. In the Ophiopluteus the right and left anterior coeloms remain separate, and the septa dividing them from the right and left posterior coeloms are formed early and are not subsequently perforated.

(*d*) A vestige of the right hydrocoele is formed in the normal Ophiopluteus larva, but in the Bipinnaria larva this only occurs as a variation.

On reading over the above comparison it is obvious that, if the fully grown larvae of *Asterias glacialis* and of *Ophiothrix fragilis* were met with for the first time, and were mistaken for adult animals, they would be regarded as genera belonging to the same family. The existence of such a deep-seated fundamental resemblance points to the former existence of a simply organized, bilaterally symmetrical, free-swimming, common ancestor of Asteroidea and Ophiuroidea. Our faith in the former existence of this ancestor will be further strengthened when we have examined the development of the three remaining classes of Echinodermata.

METAMORPHOSIS OF OPHIOTHRIX FRAGILIS

Stage A.—The metamorphosis of *Ophiothrix fragilis* is initiated, just as is that of *Asterias glacialis*, by a preponderate growth of the organs of the left side, viz. the left hydrocoele and the left posterior coelom. The former of these grows long and extends forwards, along the oesophagus, completely overlapping the left anterior coelom. At the same time it becomes divided into five lobes by constrictions which appear on its inner side. These are, of course, the rudiments of the **five radial water-vascular canals** and of the azygous **tentacles** or **tube feet** in which they terminate (Fig. 377, C). By this growth the original posterior end of the stone-canal is pulled forwards, so that it lies in front of what was originally the anterior end. A **madreporic vesicle** is formed, apparently in the same way as in Asteroidea.

The left posterior coelom also extends forwards. Its inner wall remains thin, but its outer wall begins to develop great proliferations of cells which form conical protuberances. Into each conical protuberance a narrow diverticulum of the lumen extends. These protuberances, which raise corresponding humps in the ectoderm, are the rudiments of the **adult arms.**

As the left hydrocoele increases in length its anterior end begins to extend over the dorsal surface of the stomodaeum, and its posterior end sweeps round to the right, across the ventral surface of the

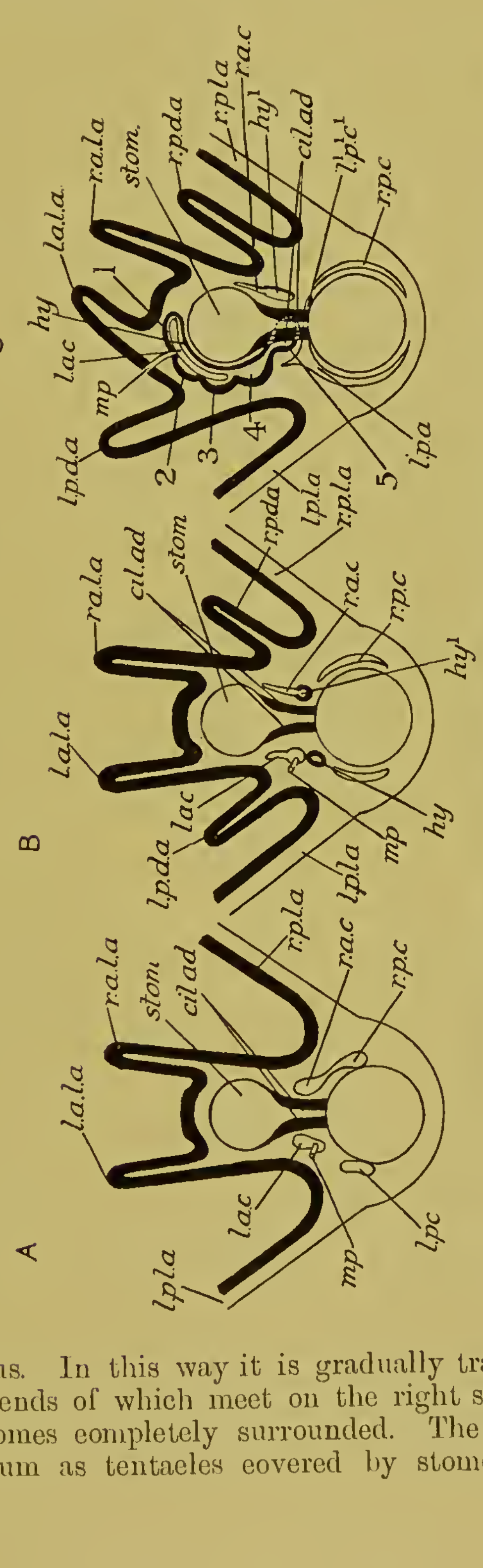

FIG. 377.—Three diagrams illustrating the internal changes which occur in the larvae of *Ophiothrix fragilis* up to the end of the first period of metamorphosis, viewed from the dorsal aspect.

A, segmentation of coelom. B, formation of hydrocoeles. C, beginning of the encircling of the stomodaeum by the left hydrocoele. *cil.ad*, adoral band of cilia; *hy*, left hydrocoele; 1-5, lobes of left hydrocoele; hy^1, rudimentary right hydrocoele; *l.a.c*, left anterior coelom; *l.a.l.a*, left antero-lateral arm; *l.p.c*, left posterior coelom; $l^1p^1c^1$, right ventral horn of left posterior coelom; *l.p.d.a*, left postero-dorsal arm; *l.p.l.a*, left postero-lateral arm; *mp*, madreporic pore; *r.a.c*, right anterior coelom; *r.a.l.a*, right antero-lateral arm; *r.p.c*, right posterior coelom; *r.p.d.a*, right postero-dorsal arm; *r.p.l.a*, right postero-lateral arm; *st*, stomach.

oesophagus. In this way it is gradually transformed into a circle, the two ends of which meet on the right side of the mouth, which thus becomes completely surrounded. The lobes project into the stomodaeum as tentacles covered by stomodaeal ectoderm. This growth of the left hydrocoele is accompanied by a growth of the whole left side of the larva. Thus we find that by this growth the left antero-lateral larval arm is carried across to the right side, so as to lie close to its right partner, whilst the left postero-dorsal arm is carried forwards to a position where it might easily be mistaken for the left antero-lateral arm (Fig. 377, C).

The left anterior coelom and its pore-canal are also carried forwards till they reach the mid-dorsal line, in other words, till they reach a position similar to that which they reach in the Asteroid larva at the same stage. The left posterior coelom sends out a **right ventral horn** ($l^1p^1c^1$, Fig. 378) which sweeps over to the right side, beneath the oesophagus, and eventually meets the horn of the same cavity which has extended dorsal to the oesophagus, thus forming a complete ring. Two of the arm rudiments are formed from this horn, the other three arise from the main part of the left posterior coelom.

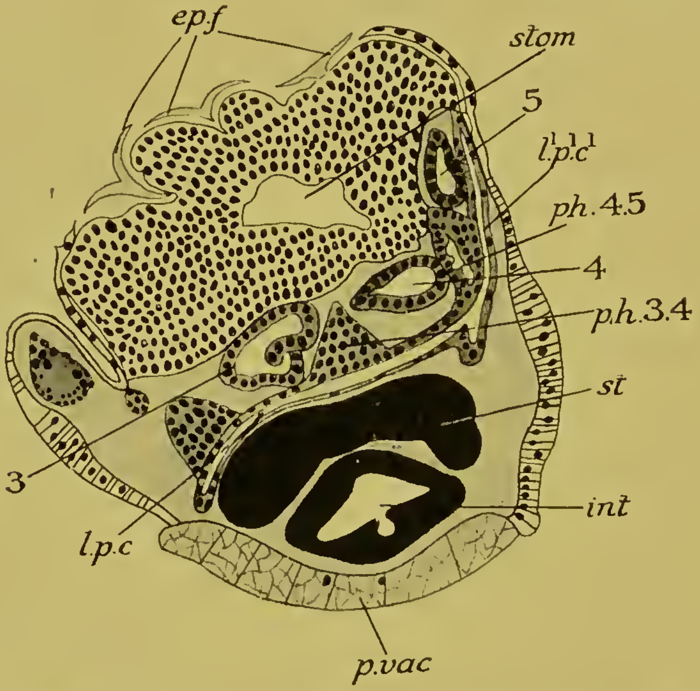

FIG. 378.—Longitudinal frontal section of a larva of *Ophiothrix fragilis*, in the first stage of metamorphosis. (Original.)

3, 4, 5, third, fourth, and fifth primary lobe of the hydrocoele respectively. *ep.f*, epineural fold; *int*, intestine; *l.p.c*, left posterior coelom; $l^1p^1c^1$, right ventral horn of the left posterior coelom; *p.h*.3.4, *p.h*.4.5, perihaemal rudiments intervening between lobes 3 and 4, and lobes 4 and 5, respectively, of the hydrocoele; *p.vac*, posterior vacuolated crest; *st*, stomach; *stom*, stomodaeum.

From the inner side of the left posterior coelom there are given off wedge-shaped, thick-walled evaginations, which alternate with the lobes of the hydrocoele and extend ventrally to them. These are the rudiments of the **perihaemal ring-canal,** and of the **radial**

perihaemal canals, which are thus formed in a manner similar to that described for *Asterias*. One of these rudiments arises from the left anterior coelom.

The main difference between these perihaemal rudiments and the corresponding structures in the Asteroid larva is, that in the Ophiuroid they are thick-walled and their cavities are mere slits (*p.h*, Fig. 378), whereas in *Asterina gibbosa*, at any rate, they are thin-walled and open by wide mouths into the coelom.

On the stomodaeal ectoderm, just over the places where these perihaemal rudiments are situated, five ridges make their appearance which radiate inwards towards the centre of the mouth. The crests of these ridges give off diverging lamellae to right and left, which may be termed **epineural flaps** (*ep*, Fig. 378). These lamellae meet those of adjacent ridges, and thus form roofs over the basal portions of the lobes of the hydrocoele, *i.e.* those portions which will form the radial canals. In this way is formed the **epineural roof** which covers in each radial nerve cord of the adult.

Stage B.—The changes which have just been described go on simultaneously, and constitute what we may call stage A of the metamorphosis. In the next stage, which may be termed stage B, further changes supervene and the absorption of purely larval organs commences.

All the larval arms, except the two postero-lateral, become reduced in size, the ectoderm covering them retreats to their bases and is devoured by phagocytes, and the spines of the larval skeleton are exposed and broken off. In the meantime the lobes of the hydrocoele each develop two pairs of lateral lobes, the rudiments of the first two pairs of tentacles representing the **paired tube feet** of Asteroidea (*b.t*, Fig. 379).

The perihaemal spaces extend outwards,—*i.e.* as in Asteroidea, the adjacent sides of two neighbouring perihaemal spaces become apposed to form the two **radial perihaemal canals** of each arm, which later fuse into one. Branches of these canals extend along the surfaces of the lateral tube feet.

The outer part of the stomodaeum, which enclosed the primary lobes of the hydrocoele, shallows out and disappears. The **adult mouth** is formed about the spot where stomodaeum and endodermal oesophagus meet one another; in a sense it is identical with the larval mouth, because it is, as it were, formed from *the deepest recess* of the structure, but it cannot be too strongly emphasized that in Ophiuroids, as in Asteroids, the stomodaeum is a temporary structure.

In the Asteroid the adult mouth is formed to the left of where the larval mouth and stomodaeum were situated. Now, in *Ophiothrix*, in this stage, the stomach and intestine are displaced *to the right*, and this is obviously the same as saying that the mouth moves to the left, because in speaking of the Asteroid the stomach and intestine are taken as fixed points. If we were to regard the outer end of the endodermal oesophagus in the Asteroid, *i.e.* the inner end of the stomodaeum, as homologous with the *adult* mouth of an Ophiuroid, then we might say that *in the Ophiuroid this point is displaced by growth towards the left side, whereas in the Asteroid it disappears and is replaced by a new growth farther to the left.* Therefore the Asteroid in this respect obviously exhibits a more modified course of events, and

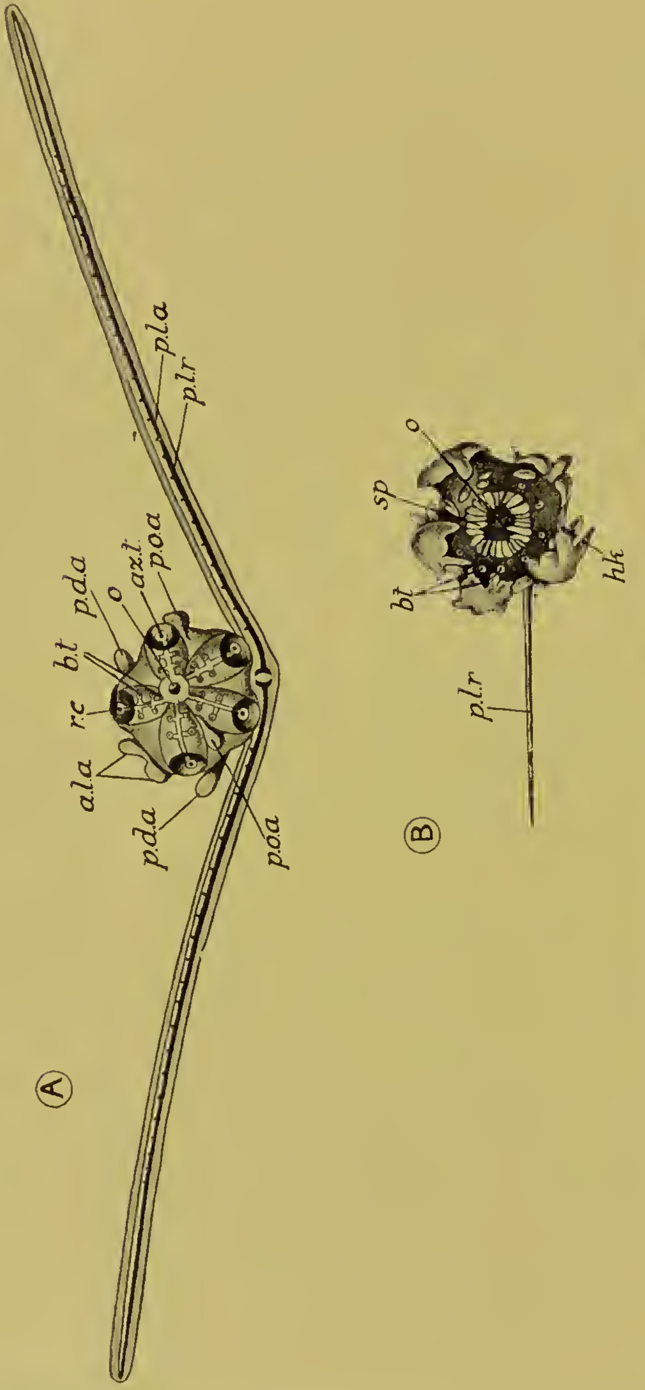

Fig. 379.—Two larvae of *Ophiothrix fragilis*, in the concluding stages of metamorphoses viewed from the ventral side. (Original.)

A, twenty-three days old. B, twenty-six days old. *a.l.a*, vestiges of antero-lateral arms; *az.t*, azygous tentacle at the end of one of the primary lobes of the hydrocoele; *b.t*, buccal tentacle; *hk*, one of the hooks on the arms of the young brittle star; *o*, adult mouth; *p.d.a*, vestiges of postero-dorsal arm; *p.l.a*, postero-lateral arm; *p.l.r*, postero-lateral rod; *p.o.a*, vestiges of post-oral arms; *r.c*, radial canal of hydrocoele; *sp*, mouth-angle spines of the young brittle star.

to this extent the development of an Ophiuroid is more primitive than that of an Asteroid.

Stage C.—In the next and concluding stage of the metamorphosis, which we may designate stage C, all trace of the larval arms, except the two postero-lateral, has disappeared. The adult arms have, however, grown in length and have become apposed to the lobes of the hydrocoele. This apposition is brought about by the shrinkage of the ectoderm connecting the two structures. The outline of the young Ophiuroid, instead of being merely pentagonal, as it was in the preceding stage, has become definitely five-rayed, but the rays are folded inwards underneath the disc.

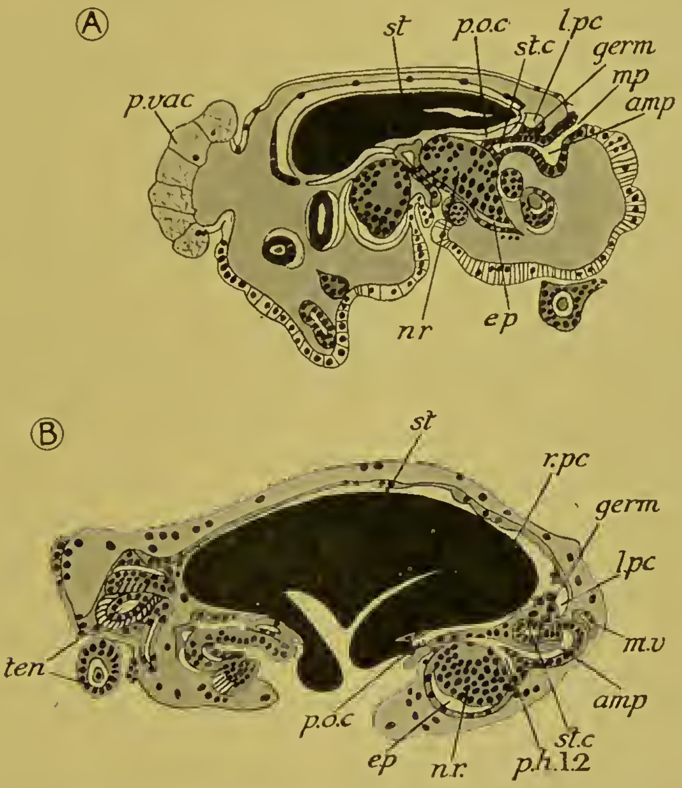

Fig. 380.—Transverse sections through the discs of two young brittle-stars in order to show the origin of the germ cells. (Original.)

A, section through young *Ophiothrix fragilis* just after metamorphosis. B, section through the embryo of *Amphiura squamata* ·25 mm. across the disc taken from the maternal brood pouch. *amp*, ampulla, the derivative of the left anterior coelom; *ep*, epineural space; *germ*, primitive germ cells; *l.p.c*, left posterior coelom; *m.p*, madreporic pore; *m.v*, madreporic vesicle; *n.r*, nerve ring; *ph*.1.2, perihaemal space, derived from the left anterior coelom (ampulla) and still opening into it; *p.o.c*, perioral coelom; *p.vac*, posterior vacuolated crest; *st*, stomach; *st.c*, stone-canal.

There is found as an outgrowth of the left posterior coelom, a **peri-oral coelom** (*p.o.c*, Fig. 380), corresponding to that found in Asteroids, which interposes itself between the left posterior coelom and the oesophagus. This appears to persist throughout life in the

Ophiuroid. In the Asteroid, as we have seen, the dividing wall between the peri-oral and the left posterior coelom breaks down and leaves as remnants the ten "retractors" of the stomach. The intestine and larval arms have by this time completely disappeared.

From the walls of the radial perihaemal spaces proliferations of cells take place. Those on the ventral side of these spaces are apposed to the thickened bands of ectoderm which form the radial nerve cords, and they constitute the **coelomic ganglia**, which are the motor elements in the nervous system. From the dorsal walls of these spaces masses of cells are given off which form the great longitudinal muscles connecting the "**vertebrae**" of the arm. From the sides of the arms **movable hooks** are developed (*h.k*, Fig. 379). These hooks are found, in the adult *Ophiothrix*, ventral to the transverse rows of arm spines, and are characteristic of the genus.

By this time the metamorphosing larva has reached the bottom, and it commences to walk on its tube feet. The postero-lateral arms shrink, the flesh retreating to their bases and the spines becoming exposed. The naked spines are soon broken off and the young brittle-star walks away. Very soon its arms have become so long that the wriggling movements, so characteristic of the adult, supersede the action of the tube feet.

AMPHIURA SQUAMATA—SKELETON AND GENITAL ORGANS

The development of the calcareous skeleton and of the genital organs has not been followed out in *Ophiothrix*, but has been worked out in the species *Amphiura squamata*. As mentioned above, this animal carries the young about in its genital bursae until they resemble the parent in all points except size and the development of the genital organs. It is hermaphrodite, one testis and one ovary discharging into each bursa.

Russo (1891) has given a general account of its development, but as this worker did not employ the method of sections to any great extent, and as the young stages are met with seldom and are very opaque, it is quite likely that his account is inaccurate. According to Russo the blastula is converted into a two-layered gastrula by delamination (!), each cell dividing into an inner endodermic and an outer ectodermic portion, and the coelom is said to arise as splits in a mass of mesenchyme. Such statements as these are improbable in the highest degree. The embryo is oval without any of the outgrowths characteristic of the Ophiopluteus; it has, however, a larval skeleton, consisting of a network of calcareous trabeculae, which is absorbed before the metamorphosis is complete. This network arises as two calcareous "stars" which arise in the mesenchyme to the right and left of the alimentary canal, and which branch, and their branches unite to form the network. Even before the pentagonal form is attained the rudiments of the adult plates begin to appear.

The most satisfactory account of the development of these plates has been given by Ludwig (1881), but Fewkes (1887) has also published a paper on the subject. We find on the aboral side of the larva a central plate, the so-called **dorso-central,** which is surrounded by five plates which are interradial in position. One of these plates surrounds the madreporic pore, which is thus at first situated at the edge of the dorsal surface, not on the oral surface, where it is found in the adult. Fewkes terms these plates **basals.** Alternating with these are found a circle of five so-called **radials,** not to be confused with the **radial shields** of the adult, a pair of which occur above the insertion of each arm. Farther out, overlying the tips of the lobes of the hydrocoele, grooved plates, concave below, represent the **terminals.** These plates protect the terminal tentacle which is formed from the tip of the primary hydrocoele lobe. Later they become converted into cylinders by the meeting of the edges of the groove.

On the oral side, alternating with the tube feet or secondary lobes of the hydrocoele, spicules of calcareous matter make their appearance. These are Y-shaped, the stem of the Y being directed towards the point of the arm and the fork towards the mouth. The fork is really the beginning of a system of dichotomous branching, by which a meshwork is produced, since the members of further dichotomies unite with one another just as has been described in the formation of an Asteroid plate. A similar process of dichotomy begins later in the end of the stem.

In this way each Y forms a narrow plate, elongated in the direction of the arm. The proximal ends of the right and left plates unite with one another first, and then the distal ends. In this way the beginning of a **vertebra** is formed. It is obvious that the two plates correspond to the ambulacral plates of an Asteroid. In the genus *Ophiohelus* the " vertebrae " consist throughout life of two rods joined at either end with a hole in the middle, just as they are in the young *Amphiura squamata.*

Other plates are formed on the sides of the arm, these are **lateral plates** or **adambulacrals,** and on the aboral side other plates make their appearance. Between dorso-central and so-called basal, on each interradius, two plates appear. These force the basal round to the ventral side of the young star-fish, and their appearance is therefore correlated with a growth in length of the interradii; the radii grow more slowly.

A plate, the so-called **underbasal,** becomes interposed between the radial and the centro-dorsal, but the radial is not forced far out or on to the ventral surface. On the contrary the greatest growth takes place between the radial and terminal, and is correlated with the growth in length of the arm. The true "**radial shields**" appear as paired plates between the embryonic radials and the terminals. The first two pairs of ambulacrals do not unite with one another, but each unites with the first adambulacral or lateral, and this forms half the jaw-frame, the other half being formed by the similar plates in the next ray.

While these developments are going on in the skeleton the foundations of the genital system are being laid. The development of this has also been worked out by us (MacBride, 1892). It will be remembered that the madreporic pore, and of course the pore-canal and left anterior coelom along with it, are forced from a marginal to a ventral position owing to the growth of the interradius. The original mesentery separating oral from aboral coeloms, *i.e.* left from right posterior coelom, is largely perforated, but it too persists and extends from the stomach obliquely downwards instead of upwards. The stone-canal also extends obliquely downwards, and where the wall of the left posterior coelom abuts on it one can observe that the nuclei of the latter become enlarged.

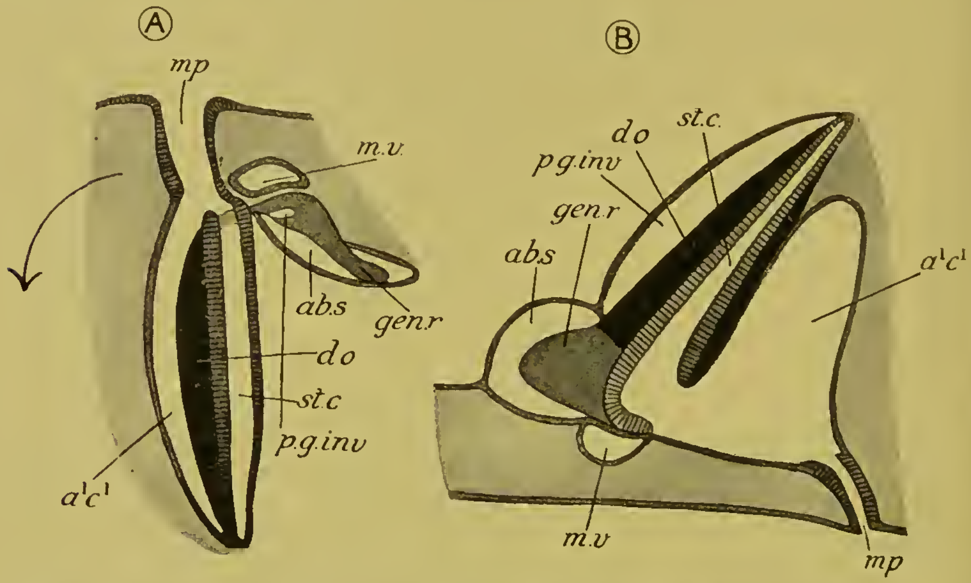

FIG. 381.—Two diagrams to elucidate the mutual relationships of stone-canal, dorsal organ, axial sinus, madreporic vesicle, genital rachis, etc., in an Asteroid and an Ophiuroid respectively.

A, relations of these organs in an Asteroid. B, relations of these organs in an Ophiuroid. *ab.s*, aboral sinus; a^1c^1, space derived from the anterior coelom—in A termed axial sinus, in B ampulla; *d.o*, dorsal organ; *gen.r*, genital rachis; *m.p*, madreporic pore; *m.v*, madreporic vesicle; *p.g.inv*, primitive germinal invagination—this space disappears in A, but in B forms the so-called axial sinus; *st.c*, stone-canal. The arrow shows the direction in which the structures in A must be rotated to reach the condition in B.

This is the first indication of the primitive germ-cells, and forms the **genital stolon** tissue, constituting the **dorsal organ.** It becomes protected by the outgrowth of a covering flap which grows down from the central end of the stone-canal. The space left between the primitive germ-cells and the cover-flap has been confused with the **axial sinus** of Asteroidea, and has received the same name; it is, however, quite distinct from this, and corresponds to the cavity of the **primitive germinal involution** of Asteroidea, which soon closes; the corresponding cavity in Ophiurids persists for life.

The **genital rachis** grows out from the peripheral end of the stolon, and the **aboral sinus** surrounding it arises by the development of a covering flap, just as happens in Asteroidea. The genital rachis pursues an undulating course, being situated aborally where it crosses the radii and ventrally in the interradii. As it slopes downward from radius to interradius it passes over the surface of the genital bursae, which develop as invaginations of the ectoderm. Here the genital organs develop as buds, which press against the bursa and form their own openings into it at maturity. Thus all the parts which can be recognized in the genital system of Asteroids are represented in Ophiuroids, but the aboral end of the stone-canal has been rotated downwards (Fig. 381).

OPHIURA BREVISPINA

The development of *Ophiura brevispina* (1900) has been worked out by Caswell Grave. The egg is comparatively large and very yolky; and development is rapid, the metamorphosis being complete in ten days. The development bears somewhat the same relation to that of *Ophiothrix* as that of *Solaster* does to *Asterias*. The earliest stages were not observed, but it appears that a blastula is formed, the cavity of which becomes filled with precociously formed mesenchyme. Then an invagination of a solid mass of cells takes place, which becomes hollowed out so as to form an archenteron, in such a way that, for a time, a solid tongue of cells projects into its cavity.

The gastrula rapidly elongates, and there is formed in front of the archenteron a long head region filled with mesenchyme. From the apex of the archenteron a coelomic vesicle is cut off, which soon divides into right and left sacs; these form the right and left anterior coeloms. At the same time the rest of the archenteron becomes cut by a circular furrow into a small dorsal sac, which forms the stomach, and large ventral one, the front part of which forms the hydrocoele and the hinder part the posterior coelom. The mouth arises as a wide shallow invagination on the ventral surface, while the hydrocoele rapidly develops its five lobes and grows round it.

The external form of the larva is now cylindrical, but slightly thicker behind than in front. The universal coat of cilia has given place to five transverse rings of cilia, which give the creature the appearance of being segmented and led Johannes Müller, by whom it was first discovered, to call it the "**worm-like larva.**" It glides slowly over the bottom. Soon the hydrocoele ring becomes completed and the azygous tentacles appear as external protrusions, and in this way a pentagonal figure is outlined on the under side of the posterior part of the larva.

Although the hydrocoele arises from a rudiment common to the left posterior coelom, it becomes cut off from this and forms a secondary connection with the left anterior coelom, which is the **stone-canal.** Only after this has occurred is the pore-canal formed. The larva now looks like a young Asteroid, and for this reason Johannes Müller included the "worm-like larva" amongst the Asteroid larvae.

As development proceeds the anterior part of the larva diminishes in size, and the adult arms grow out. The epineural spaces appear at first as open grooves, the edges of which meet. The perihaemal spaces arise exactly as in *Ophiothrix.*

The only strikingly abnormal features about this development are the method of the gastrulation and of the formation of the coelom. With regard to the first point, it is a remarkably interesting fact that if the eggs of *Ophiothrix* be removed from the ovary and artificially fertilized, instead of being allowed to be spawned naturally, they often exhibit the same kind of early development as Grave has described for *Ophiura brevispina.* The mesenchyme begins to be cut off in the early stages of segmentation, so that the blastocoele, from its very beginning, is filled with it, and the blastula is in reality a "morula." Invagination is a solid inwandering of cells, and, when this solid mass becomes hollowed out, a tongue of cells persists which projects into the lumen of the archenteron. Such larvae never develop the anterior vacuolated crest.

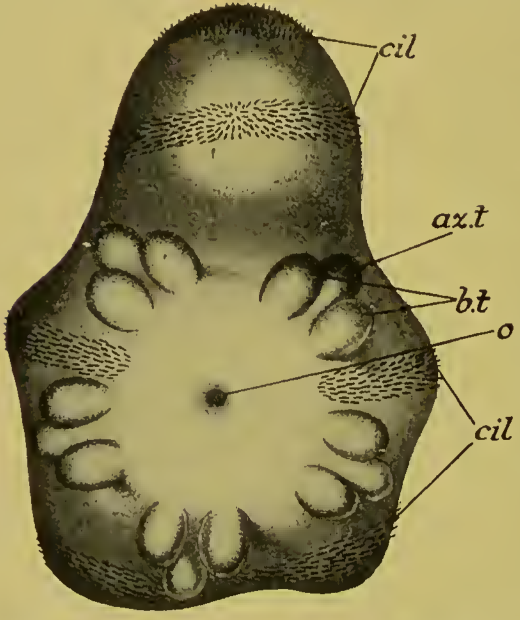

FIG. 382.—Ventral view of the larva of *Ophiura brevis* during the metamorphosis. (After Grave.)

az.t, rudiment of azygous tentacle; *b.t*, rudiments of buccal tentacles; *cil*, transverse ciliated bands; o, mouth.

This interesting fact leads us to form some such conclusion as this. The processes of growth and cytotaxis, which are the underlying causes of the outward and visible "signs" of formation of mesenchyme, invagination, etc., are the same over a wide range of species. The relations of these processes to each other in *degree of intensity* and *times of maximal intensity*, in a word *the differential equations which connect them,* differ as we pass from species to species, and in consequence the same processes give rise to very different visible results in different cases. By interfering with the normal relations in any one species, in the case of *Ophiothrix,* by fertilizing the egg before it is quite ripe, the conditions usually found in quite a different species may be reproduced.

With regard to the formation of the coelom, we have learnt how profoundly this process may be altered amongst Asteroidea by the presence of yolk, and so we are the less surprised at its abnormality in *Ophiura*. But it should be added that Grave had only a limited amount of material to work on, which he could not augment as second attempts at artificial fertilization did not succeed. Hence it is possible that some of his interpretations may be mistaken.

DEVELOPMENT OF OPHIOTHRIX AND ASTERIAS COMPARED

When we compare the development of *Ophiothrix* with that of *Asterias* we notice two main differences: first, the fixed stage is dropped out completely; the Ophiuroid, by the retention of the postero-lateral larval arms, is enabled to pass through its metamorphosis floating; and, secondly, some of the larval changes are simpler and more direct in character than in *Asterias*. Thus the segmentation of the coelom is not complicated by subsequent perforations of the septum, and the larval mouth persists. The perforations of the septum seem to be due to the body musculature developed in Bipinnaria, which renders it necessary to allow the coelomic fluid to pass from one part to another. It is hardly likely that, in the primitive condition of affairs, if a segmentation took place it would be largely undone immediately afterwards, so that in this respect *Ophiothrix* is probably more primitive than *Asterias*.

Then, as to the retention of the larval mouth in the adult, there can be no question that this more truly represents the history of the race than the obliteration of the old mouth and the formation of a new one in a new place. It is not easy to picture an animal in the condition of losing an old mouth and gaining a new one. We have seen that when the hydrocoele ring is just complete there is no epineural fold developed, so that the nerve cord is exposed; and further, that when metamorphosis is just complete the animal walks on its tube feet instead of using its arms. Young specimens of *Amphiura squamata*, if cut out of the bursa of the parent when the arms are as yet undeveloped, walk also on their tube feet, so that in these two respects the Ophiuroid may be said to pass through an Asteroid stage.

Now there are found in Silurian and Devonian rocks a considerable number of fossils exactly intermediate in character between Asteroidea and Ophiuroidea. We have every reason to believe that in these fossils we possess the actual record of the evolution of the Ophiuroidea, and we are therefore in a position to test how far the history of the individual, as disclosed by embryology, agrees with the history of the race. Now these fossils show an open ambulacral groove, and ambulacral ossicles not yet united to form vertebrae, but in the larva the open ambulacral groove becomes closed long before there is any trace of vertebrae, and while the adult arms are still mere stumps.

Hence we conclude that in the larval history there has been a *dislocation of the sequence of events,* and that the formation of the epineural flaps has been hurried on long before its time—in a word, that these flaps have been precociously developed. In dealing with the development of Gastropoda, it will be remembered we saw reason to believe that, in the veliger stage, we had an example of the opposite phenomenon, viz. the retention of a larval organ long beyond the stage when, according to ancestral history, it ought to have disappeared.

ECHINOIDEA

The complete life-cycle has been described in a very few Echinoidea. We possess records of the external appearance of the larva in all stages of development in the case of *Echinus miliaris* and *Echinus esculentus*, two species of regular Endocyclic urchins; also in the cases of *Echinocyamus pusillus* and *Mellita testudinata*, two Clypeastroids; and of *Echinocardium cordatum*, a Spatangoid. In the case of *Echinus esculentus* the development has been thoroughly investigated by us by means of sections (MacBride, 1903), and we shall select this species as a type for detailed description.

Owing to the fact that the ovaries and testes in Echinoidea are larger in proportion to the body and more compact than in any other class of Echinodermata, a very large proportion of the experiments, on which the science of "Experimental Embryology" is founded, have been performed on the eggs of Echinoidea. With hardly an exception, however, the experimenters have confined their attention to that part of the development which terminates in the formation of the four-armed larva; and further, the overwhelming majority of the experiments have been performed on the three species most commonly found at Naples, viz. *Echinus microtuberculatus*, *Strongylocentrotus lividus*, and *Sphaerechinus granularis*. All three are regular urchins, and, in all important points, all three have given identical results in the hands of experimenters.

When we reflect that *Echinus microtuberculatus* is only a local race of *Echinus miliaris*, and that, except for insignificant details, the early development of *Echinus miliaris* and *Echinus esculentus* is the same, and that the development of *Strongylocentrotus lividus*, which has recently been investigated by von Übich (1913), seems to be in all respects similar to that of *E. miliaris*, we shall, without hesitation, apply to *Echinus esculentus* the minute knowledge of the earliest stages of development which has been gained by the concentration of the attention of the experimenters on the Neapolitan species.

We shall first describe the normal development and then briefly sketch the principal results arrived at by experiment.

The eggs of *Echinus esculentus* can be made to develop through their complete life-cycle even when artificially shaken out of the ovary. It is by no means necessary to wait for a natural spawning in order to get good results. *Echinus esculentus* is a gregarious form and can usually be obtained in large numbers, either by dredging at moderate depths, as in Plymouth Sound, or by picking it off the rocks at low tide in more sheltered situations, as in the Clyde.

On the east coast of North America we find the species *Strongylocentrotus drocbachiensis* under similar conditions in countless thousands, and as the larva of *Strongylocentrotus* cannot be distinguished from the larva of *Echinus* by any certain mark, all that will be said about *Echinus esculentus* may be taken as applying to *Strongylocentrotus*. On the Pacific coast *Strongylocentrotus drocbachiensis* is replaced by the larger species *S. purpuratus* and *S. franciscanus,* the eggs of which are equally suitable for rearing.

METHOD OF OBTAINING NORMAL EMBRYOS

The urchins are most easily opened by inserting one blade of a pair of scissors through the margin of the peristome, and then cutting along a meridian of the shell till the equator is reached; the cut is then carried completely round the equator and so the shell is separated into an upper and a lower half. In the upper half will be found the genital organs.

The ripeness of the testes can be ascertained by squeezing out a drop of the spermatic fluid into a drop of sea-water, when a glance at the mixture through the low power of a microscope will show whether the spermatozoa are active or not.

The ripeness of the eggs can be gauged by two criteria: (1) whether they break loose from the ovary at the slightest touch, so that the ovary appears to dissolve into dust; (2) whether the large germinal vesicle, so characteristic of the unripe egg, has dissolved. Bolton cloth of a mesh of $\frac{1}{5}$ mm. is a useful adjunct. If a piece of ovary be wrapped in a piece of this cloth and shaken into clean sea-water, the eggs, which easily detach themselves, will escape through the meshes of the cloth, and the younger undeveloped ova and follicle cells, which adhere to one another, will remain behind.

A better method of obtaining ripe eggs, however, is to invert the upper half of the bisected urchin in a small glass dish containing some sea-water. The ripe eggs will then be discharged practically without admixture of unripe ones, an admixture which it is impossible to avoid when pieces of the ovary are shaken out into sea-water.

If the eggs are perfectly ripe they will be free from all membranes; but they show a thick glassy chorion, just like that which we have described in the eggs of *Asterias glacialis,* which prevents the easy access of spermatozoa. If, however, these eggs are allowed to lie for two hours in clean sea-water before being fertilized, the chorion will completely disappear. Fertilization can, however, take place before the chorion disappears.

In fertilizing, care must be taken that the eggs are spread out over the bottom of the vessel in a single layer, and that a very small quantity of spermatozoa is added. In practice, an emulsion of spermatozoa is prepared by shaking up a drop or two of thick spermatic fluid with sea-water in a specimen tube four or five inches long. If the eggs are contained in a glass evaporating dish of the usual size (say eight inches in diameter), it is sufficient to add half a dozen drops of this emulsion to ensure fertilization of every egg, if the mixture be thoroughly stirred up.

When once the eggs have fallen to the bottom, the supernatant fluid, which contains the superfluous spermatozoa, must be decanted off. Fresh sea-water is then added, and the eggs stirred up in it, and the decantation repeated. In this way all the unused spermatozoa are removed; if this is not done they die and befoul the water and impede the development of the eggs. Whether the fertilization has been successful or not can be determined by examining a sample of the eggs under a microscope half an hour afterwards. It will be then seen that all the eggs which have been ready to receive a spermatozoon have formed fertilization membranes, between which and the egg there is an accumulation of fluid.

In eighteen to twenty-four hours the larvae will have burst these membranes, and will be found swimming at the surface of the water. They can then be decanted off into vessels filled with sterilized sea-water (prepared as described under Asteroidea), and supplied with food in the form of a pure culture of the diatom *Nitschia,* when they will metamorphose into young sea-urchins in a period varying from six weeks to two months. Too many larvae must not be reared in one jar. In practice it is found that twenty or thirty is the largest number which will thrive in a half-gallon jar. Direct sunlight is to be avoided, and the larvae seem to do better when contained in a jar made of green bottle-glass.

The methods of preservation, both for whole mounts and sections, are the same as those prescribed for the larvae of *Asterias* and *Ophiothrix.*

ECHINUS ESCULENTUS

The egg divides into two and then four equal cells, and the 8-cell stage is reached by the division of the four cells into two tiers of four each. These cells touch each other laterally; eventually they are separated from one another by a space, the **blastocoele.** As development goes on the blastocoele enlarges.

Then the 16-cell stage follows. This is reached by the members of one tier dividing, each into a very small cell below, termed the **micromere,** and a larger one above, the **macromere.** Each of the members of the other tier divides by radial cleavage into two equal cells, and so a circle of eight cells of intermediate size is formed. These are termed **mesomeres.** Driesch's experiments (1900) have demonstrated what Boveri showed to be the case by direct observation in the egg of *Strongylocentrotus lividus* (1901), viz. that the micromeres are situated at the vegetative pole of the egg, not at the animal pole as had been previously imagined.

In the next cleavage each micromere buds off a smallest micromere, and the other cells divide into daughters of equal size. In subsequent cleavages each of the smaller micromeres divides once and then stops, whilst each of the larger micromeres divides three times, giving eight cells. Each macromere and each mesomere divides five times, giving in each case sixty-four cells.

When these divisions have been accomplished each cell acquires a cilium, and the hollow sphere of cells begins to rotate within the egg-membrane, which it soon bursts. It then rises to the surface of the water as a free-swimming **blastula.**

If the description of the cleavage of the egg, which has just been given, has been correctly followed, it will be seen that the blastula consists of 4 × 2 (smaller micromeres) + 4 × 8 (larger micromeres) + 4 × 64 (macromeres) + 8 × 64 (mesomeres) cells, *i.e.* 808 cells altogether. The blastula soon becomes flattened at the vegetative pole, and the cells here begin to divide and to give off **primary mesenchyme** into the blastocoele, just as happens in the case of the larva of *Ophiothrix fragilis.* This mesenchyme originates, almost certainly, from the descendants of the smaller micromeres. Only a limited number (fifty or thereabouts) of these mesenchyme cells are formed; they arrange themselves in a ring round the periphery of the blastocoele, the plane of which is parallel to the plane formed by the vegetative surface.

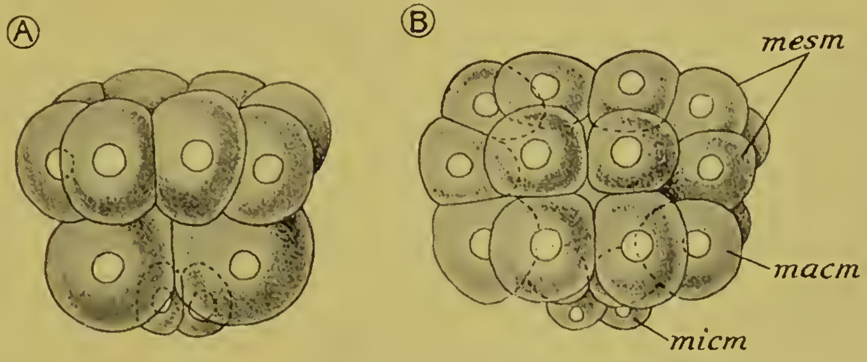

FIG. 383.—Two stages in the segmentation of the egg of *Strongylocentrotus lividus.* (The segmentation of the egg of *Echinus esculentus* pursues an identical course.) (After Boveri.)

A, 16-cell stage. B, 32-cell stage. *macm*, macromere; *mesm*, mesomere; *micm*, micromere.

In two places, diametrically opposite to each other, the ring is thickened and consists of a heap of cells; elsewhere it consists of a single line of them connected with each other by pseudopodia. In the centre of each heap a little **triradiate calcareous spicule** appears; these heaps of cells and their contained spicules are the first organs which indicate the future bilateral symmetry of the larva.

Then the blastula becomes a **gastrula** by the invagination of the archenteron, which begins in the centre of the flattened surface. As in *Ophiothrix* and *Asterias* the **archenteron** is a narrow tube of small diameter compared with the gastrula, but it reaches nearly to the anterior end of the larva. No vacuolated crests of any kind are developed, but with this exception the subsequent development, up to the age of six days, is, point for point, identical with that of *Ophiothrix*. The separation of the coelom from the archenteron, its division into two sacs, the formation of the madreporic pore, the formation of the stomodaeum, the differentiation of the gut, the formation of the adoral band of cilia, and of the longitudinal locomotor band of cilia, of the first four larval arms, and of their supporting calcareous rods, take place in precisely the same way as in the Ophiopluteus larva and need not be specially detailed here.

No wonder that the Echinoid larva, when discovered by Johannes Müller, was regarded by him as another species of his genus "*Pluteus*." It is now termed Echinopluteus in order to distinguish it from the Ophiuroid larva. The sole differences between the two which can be detected at this stage concern—(1) The posterior ciliated arms; these are more closely approximated to one another than in the Ophiopluteus, and, as subsequent development shows, correspond to the **post-oral** arms of that larva, not to those which are just formed in the Ophiopluteus, *i.e.* the postero-lateral. The Echinopluteus in this stage is consequently rather more square in section—not so flattened, in fact, as the Ophiopluteus. (2) The body-rods; these, instead of ending in the aboral pole in bifurcations, as is the case in the Ophiopluteus larva, end in inbent crooks, which may branch slightly but which do not unite with their fellows on the opposite side of the body.

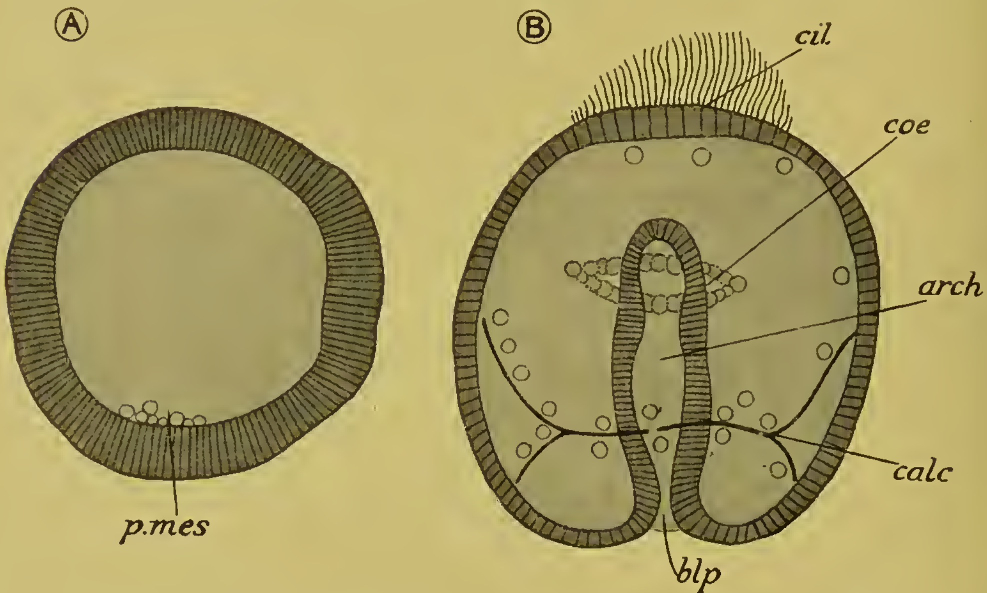

FIG. 384.—Early stages in the development of *Echinus esculentus*. (Original.)

A, blastula. B, advanced gastrula, showing the formation of the coelom. *arch*, archenteron; *blp*, blastopore; *calc*, calcareous star; *cil*, apical tuft of specially long cilia; *coe*, coelomic vesicle arising as an outgrowth from the archenteron; *p.mes*, primary mesenchyme cells.

In a well-developed larva of this age, the body consists of a conical mass which contains the larval stomach and intestine, and from which spring the post-oral arms, and of an **oral lobe** containing the mouth and oesophagus, from which spring the antero-lateral arms.

The development during the second week of larval life presents many resemblances to the development of the Ophiopluteus. Thus the coelomic sacs divide into anterior and posterior portions, the latter applying themselves to the stomach and the former to the oesophagus, to which they supply constrictor muscles. A pair of **postero-dorsal** arms are developed, but these, unlike their homologues in the Ophiopluteus, are supported by calcareous rods developed from spicules totally independent of the primary calcareous spicules.

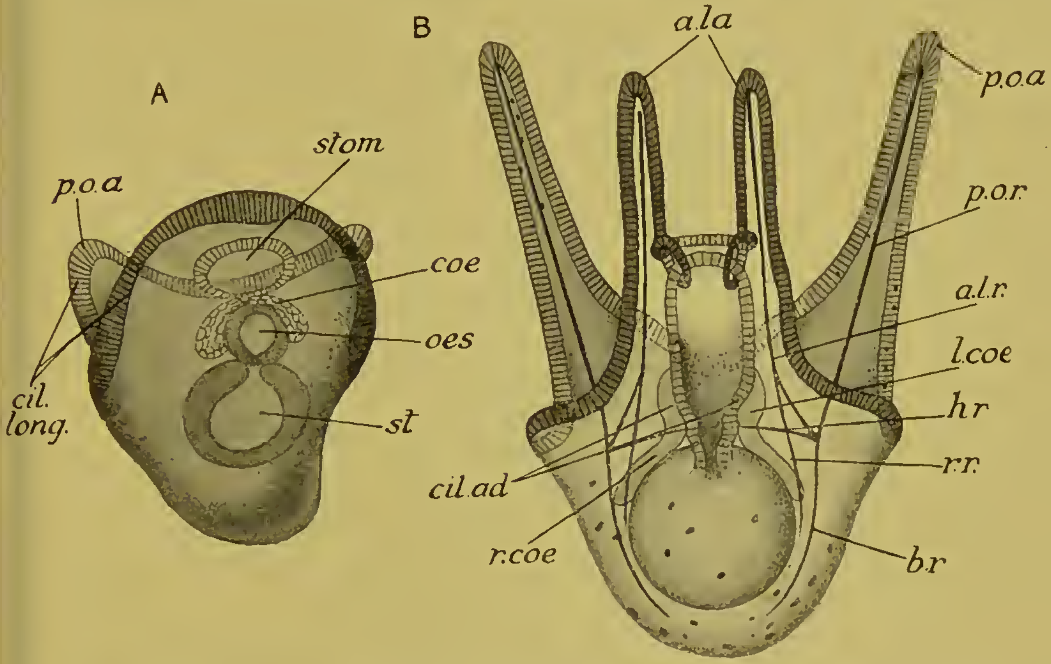

Fig. 385.—Young larvae of *Echinus esculentus* viewed from the dorsal surface. (Original.)

A, prism larva, three days old. B, four-armed Echinopluteus larva, seven days old. *a.l.a*, anterior lateral arm; *a.l.r*, antero-lateral rod; *b.r*, body-rod; *cil.ad*, adoral band of cilia; *cil.long*, longitudinal band of cilia; *coe*, coelomic vesicle; *h.r*, horizontal rod; *l.coe*, left coelomic sac; *oes*, oesophagus; *p.o.a*, post-oral arm; *p.o.r*, post-oral rod; *r.coe*, right coelomic sac; *st*, stomach; *stom*, stomodaeum.

About the end of the second week rudiments of a fourth pair of arms appear; these are situated at the sides of the loop of the longitudinal ciliated band which overhangs the mouth, the prae-oral loop in fact. These are the **prae-oral** arms; they are supported by the forks of an independent V-shaped calcareous centre termed the **dorsal arch,** which is situated in the mid-dorsal line above the region of the oesophagus.

The larva has thus five independent calcareous centres and eight arms. These arms are capable of movement, and can be slowly approximated or divaricated. These movements are due to muscular strands connecting the rods near the aboral pole, and to others going out to them from the ends of the oesophagus. Both sets are derived from cells of the secondary mesenchyme.

When it is about twelve days old the left anterior coelom buds off a posterior vesicle, which remains connected with it by a narrow neck. The vesicle is the **hydrocoele** which gives rise to the adult water-vascular system, and the neck is the stone-canal. Where this neck joins the anterior coelom the latter is dilated, and this dilatation is the rudiment of the **axial sinus** of the adult.

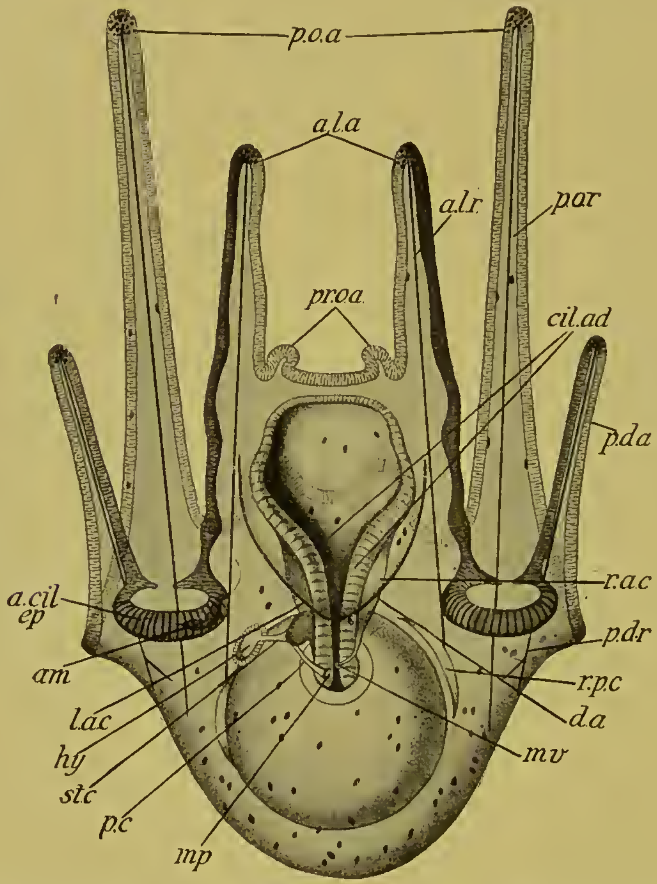

Fig. 386.—Echinopluteus larva of *Echinus esculentus* about eleven days, viewed from the dorsal surface to show the formation of the ciliated epaulettes. (Original.)

Names as in previous figure. In addition, *a.cil.ep*, anterior ciliated epaulette; *am*, amniotic invagination; *d.a*, dorsal arch; *hy*, hydrocoele; *l.a.c*, left anterior coelom; *l.p.c*, left posterior coelom; *m.p*, madreporic pore; *m.v*, madreporic vesicle; *p.c*, pore-canal; *p.d.a*, postero-dorsal arm; *p.d.r*, postero-dorsal rod; *pr.o.a*, prae-oral arm; *r.a.c*, right anterior coelom; *r.p.c*, right posterior coelom; *st.c*, stone-canal.

At about sixteen days the right anterior coelom buds off a solid mass of cells which, for a short time, remains connected with it by a string of cells; this mass of cells hollows out to form a vesicle, the **madreporic vesicle**, but the string of cells vanishes, and the right coelom never exhibits a dilatation corresponding to that of the axial sinus on the left side (*m.v*, Fig. 386).

About the same time the longitudinal ciliated band becomes thickened in four places, and bent outwards in the form of four horizontal semicircular loops. Of these loops two are situated, one on each side in the re-entrant angle of the ciliated band, between the antero-lateral and postero-dorsal arms, and these are termed the **dorsal ciliated epaulettes**; and two, one on each side on the course of the posterior cross-bar, which are termed the **ventral ciliated epaulettes.**

These four ciliated epaulettes, distinguished as the **anterior ciliated epaulettes**, become separated from the rest of the band, which heals the breaches thus made in its continuity by special growth from the broken ends (Fig. 386). The four ciliated epaulettes then grow till they almost if not quite meet each other in the mid-dorsal and mid-ventral lines; they form the main locomotor organ of the later larva, on which they bestow something of the aspect of a tiny medusa; they are much thicker, and carry much more powerful cilia than the rest of the band. The aboral ends of all the skeletal rods undergo resorption

ECHINOPLUTEUS AND OPHIOPLUTEUS LARVAE COMPARED

Leaving aside the ciliated epaulettes, which are by no means universal in Echinoid larvae, and so far as yet known are confined to the larvae of the genera *Echinus* and *Strongylocentrotus*, we may pass in rapid review the chief differences between the Echinopluteus and Ophiopluteus larvae at the height of their respective developments.

The Ophiopluteus has only two calcareous centres for the larval skeleton, one of which supplies the skeleton for all the larval arms of one side. The Echinopluteus has five calcareous centres, of which the two primary supply the antero-lateral and post-oral arms on each side, the two lateral supply the postero-dorsal arms, and the median dorsal supplies the two prae-oral arms.

The Ophiopluteus has no prae-oral arms, and possesses a pair of postero-lateral arms, which are developed first and which are the main organs of locomotion. The Echinopluteus has prae-oral arms, but the post-oral arms are developed first, and when postero-lateral arms are developed (they are absent in the larva of *Echinus esculentus*, but appear in the larvae of the Spatangoids) they appear very late, and are the shortest of all the arms.

It is obvious that the differences so far enumerated are of minor importance, and would be such as one would expect to find separating the larvae of two allied families. It is when we come to consider the metamorphosis of the Echinopluteus larva that the radical differences between it and the Ophiopluteus become apparent.

METAMORPHOSIS OF THE ECHINOPLUTEUS

The metamorphosis may be said to begin about the fifteenth day, when the ciliated epaulettes have been formed and the madreporic vesicle is making its appearance. The hydrocoele has now the form of a vesicle of a somewhat flattened oval shape when seen in cross-section, with a wall consisting of a single layer of columnar cells. On the left side of the larva, between postero-dorsal and post-oral arms, an invagination of the ectoderm can be seen above the spot where the left hydrocoele is situated. This invagination is termed the **amniotic invagination**; it is lined by cubical cells, which contrast with the flattened cells of the general ectoderm on the one hand and the highly columnar cells of the hydrocoele on the other. The amniotic invagination grows inward till its floor becomes flattened against the wall of the hydrocoele.

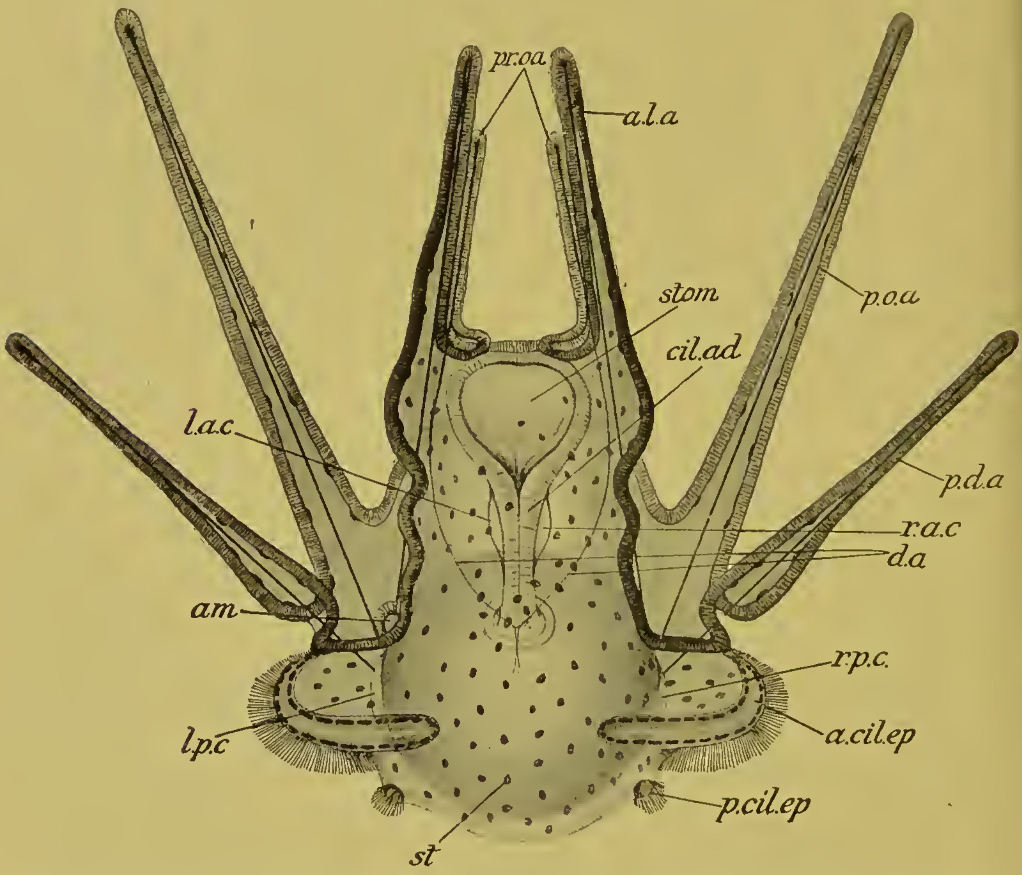

Fig. 387.—Echinopluteus larva of *Echinus esculentus* twenty-three days old, viewed from the dorsal surface. (Original.)

Names as in previous figure. In addition, *p.cil.ep*, posterior ciliated epaulette.

As a consequence of the changes which these two adpressed organs undergo, the characteristic oral disc of the young sea-urchin is built up; and the compound structure composed of these two organs is conveniently termed the **Echinus rudiment**, though it must never be forgotten that most of the body of the larva, and not merely the Echinus rudiment, is incorporated in the body of the young sea-urchin.

The hydrocoele, seen from the side, appears at first like a circular disc; soon, however, a slight notch appears in its posterior border, the two ends of this notch join, and so the disc becomes a ring. Before the ring form is

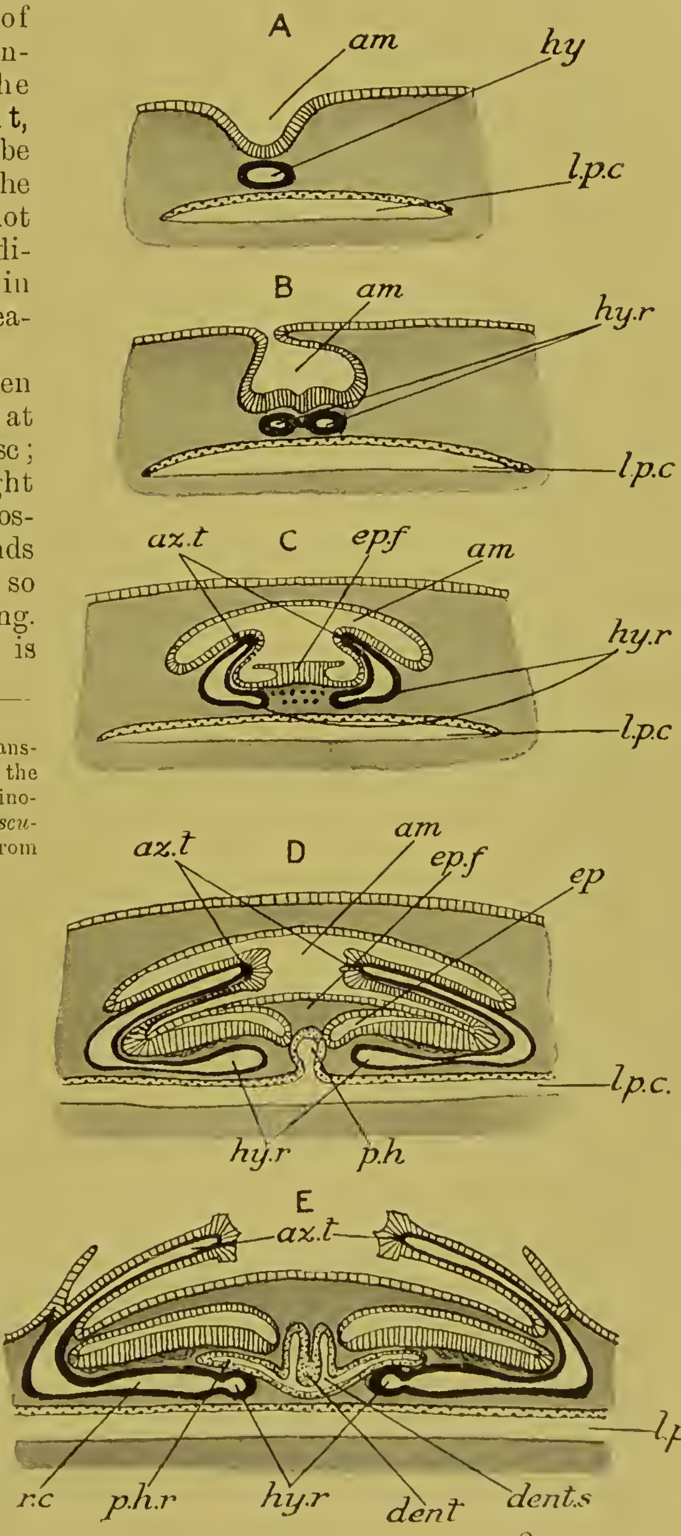

Fig. 388. — Diagrammatic transverse sections through the "Echinus rudiment" of Echinoplutei larvae of *Echinus esculentus* ranging in age from twenty-one to fifty days.

A, Echinus rudiment just formed by the apposition of the amniotic cavity and the hydrocoele. B, hydrocoele a ring; amniotic cavity closing. C, amniotic cavity closed; primary tube feet of hydrocoele protruding into it—epineural folds forming. D, epineural folds united, covering over epineural cavity—perihaemal rudiment formed. E, amniotic roof burst, and primary tube feet protruding—perihaemal rudiment developed into dental sac and radial perihaemal canal. *am*, amniotic invagination and cavity; *az.t*, primary tube foot of hydrocoele; *dent*, rudiment of tooth; *dent.s*, dental sac; *ep*, epineural cavity; *ep.f*, epineural fold; *hy*, hydrocoele; *hy.r*, hydrocoele ring; *l.p.c*, left posterior coelom; *p.h*, perihaemal rudiment; *p.h.r*, perihaemal radial canal; *r.c*, radial canal of the water-vascular system.

complete, therefore, the hydrocoele has the form of a hoop, recalling its form in *Asterias* and in *Ophiothrix* after metamorphosis has commenced. The opening of the amniotic invagination becomes first narrowed and then completely closed, so that it is converted into a sac with a thick floor which is incorporated with the Echinus rudiment, and a thin roof which is the **amnion.**

The subsequent course of the metamorphosis consists mainly in the enlargement of the Echinus rudiment. The hydrocoele soon sends out five blunt lobes which protrude as finger-shaped processes into the amniotic cavity. These are the rudiments of the five radial water-vascular canals, and of their primary terminal azygous tube feet (*az.t*, Fig. 388). In the intervals between them there are formed five radiating ridges of ectoderm which are termed the **epineural ridges.** The crests of these form two diverging lamellae, which fuse with those of adjacent ridges and so roof in the **epineural canals** covering the bases of the tentacles. These epineural canals meet in a central **epineural space,** roofed over by a membrane termed the **epineural veil,** which forms an upper floor to the amniotic cavity.

From the left posterior coelom, about the twenty-fourth day, there are given off five pocket-shaped evaginations situated one under the origin of each epineural ridge, into the substance of which it projects. These evaginations are the homologues of the perihaemal pockets of the Asteroid and Ophiuroid larvae; they *all* originate from the left posterior coelom, while it will be remembered that in the two other larvae mentioned, one originates from the left anterior and four from the left posterior coelom. About the same time it can be observed that the left posterior coelom has sent out right dorsal and right ventral horns. They, however, are of small extent in comparison with the bulk of the vesicle; they soon meet each other anteriorly and so a ring is formed, surrounding, not the oesophagus, but the stone-canal.

The Echinus rudiment as thus described forms at first a comparatively small, star-shaped organ on the side of the larva, but as development proceeds it becomes ever larger till it occupies the whole side of the larva. This consummation is arrived at about the fortieth day.

The **adult central nervous system** is formed, of course, from the ectoderm immediately covering the hydrocoele, and forming the lower floor of the amniotic cavity. It can be first detected by a thickening of this ectoderm and a great increase in the nuclei; this occurs as early as the twenty-first day. Later, fine fibrils can be detected at the base of the ectoderm and can be traced into some of the nuclei.

As the Echinus-rudiment increases in size, the tube feet become longer and longer and project farther into the amniotic space. **Pointed spines** make their appearance, rising from the upper floor of the amniotic cavity. There are four spines in each interradius arranged in the form of a lozenge. Each of these spines is a hollow conical outgrowth of the ectoderm of the upper floor of the amniotic cavity. It is filled with mesenchyme cells which have been budded from the wall of the left posterior coelom. These cells arrange themselves in a network, in the interstices of which appears calcareous matter which consequently assumes the form of a lattice-work—a negative, so to speak, of the framework of cells to which it owes its origin. The bases of these spines are thickened so as to look like collars, and here the contained mesenchyme undergoes transformation into the muscles which attach the spine to its boss. The overlying ectoderm develops nervous fibrils.

The perihaemal pockets begin to send off narrow outgrowths between ectoderm and outgrowth of hydrocoele, and form the two **radial perihaemal canals** in each radius, which, in the adult, fuse into one (*p.h.r*, Fig. 388). The body of each perihaemal space remains larger than it does in the Asteroids and Ophiuroids, and on its outer wall a protrusion appears which projects into its cavity. This protrusion is the formative tissue for one of the **teeth,** and the body of the perihaemal pocket into which it projects forms one of the five **dental sacs** which, collectively, form the cavities of **Aristotle's lantern.**

It is characteristic of the later stages of metamorphosis that the cavity of the hydrocoele ring becomes enormously distended with fluid. In the centre of this ring there appears an invagination of the ectoderm which constitutes the lower floor of the amnion. This invagination is the **adult stomodaeum,** a structure which does not exist in Asteroidea and Ophiuroidea; a small peg-like outgrowth protrudes from the stomach towards it, which eventually meets the adult stomodaeum and the two fuse, and so the adult mouth is formed.

If this description has been followed it will be seen that the adult mouth is at first shut off from the exterior, not merely by the roof of the amniotic cavity but also by the epineural veil. The swelling of the Echinus rudiment has indented the larval stomach; it is no longer globular, but hemispherical, a flattened side being turned towards the Echinus rudiment.

Whilst these changes have been occurring and the Echinus rudiment has been growing in size, other changes have been occurring in other parts of the larva. From the tips of the re-entrant angles of the ciliated band, which are situated between the postero-lateral and post-oral arms, a pair of ciliated epaulettes become separated off (*p.cil.ep*, Figs. 387 and 390). This extra pair may be termed the **posterior ciliated epaulettes.** They are characteristic of the larva of *Echinus esculentus*; they do not appear in the larva of *E. miliaris.* They make their appearance about the twenty-fifth day.

At the same time three **pedicellariae** of the **ophicephalous** type make their appearance on the larva; each arises as a little knob-like outgrowth of the ectoderm, into which mesenchyme cells are budded from an adjacent area of the coelom. Some of these cells form the skeleton of the organ and some the muscles. One of these pedicellariae is situated near the aboral pole of the larva; this one is not developed in the larva of *E. miliaris.* At the base of this aboral pedicellaria a plate is developed. The other two pedicellariae are situated on the right side of the larva, and are supported by a single plate. A third plate begins to be formed around the madreporic pore; this is the beginning of the madreporite. All three plates belong to the series of **basals** which we have already encountered in the Asteroid and Ophiuroid, and which, as shown by Bury (1895), form the **genital plates** of the adult. Small spicules, rudiments of the remaining two plates, can now be detected.

Whilst these changes are occurring, the right and left posterior coeloms become dilated, approach one another, and fuse at the aboral pole of the larva; and from the wall of the left posterior coelom, where it abuts on the hinder wall of the left anterior coelom, a solid bud of cells grows out and buries itself in the blastocoele which intervenes between the stomach and the inner wall of the left posterior coelom. This is the rudiment of the **genital stolon.**

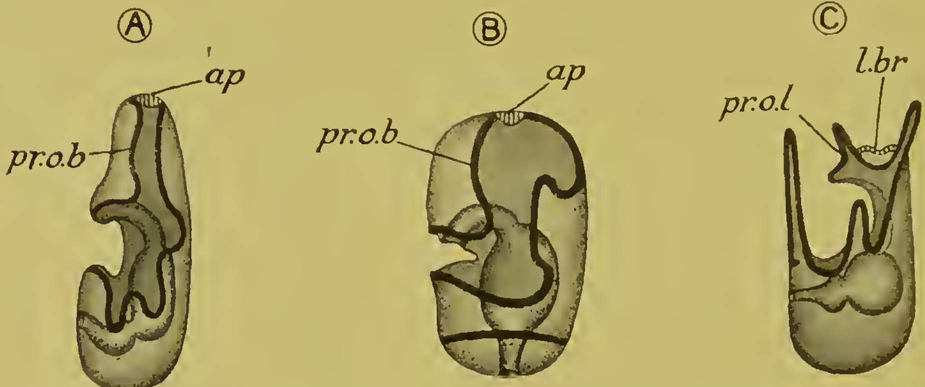

FIG. 389.—Diagrammatic side-views of larvae of an Asteroid, Echinoid, and Balanoglossid, to show the comparability of the apical plate of an Asteroid with the larval brain of the other two types of larvae.

A, diagram of Bipinnaria larva, viewed from the side. B, diagram of Tornaria larva of Balanoglossid, viewed from the side. C, diagram of Echinopluteus larva, viewed from the side. *ap*, apical plate ; *l.br*, larval brain ; *pr.o.b*, prae-oral band of cilia ; *pr.o.l*, prae-oral loop of longitudinal ciliated band.

About the twenty-fourth day a remarkable **larval nervous system** makes its appearance. It arises as a shallow ectodermal pit on the dorsal aspect of the oral lobe, in the middle line behind the anterior cross-bar of the longitudinal ciliated band. The ectodermal cells lining this pit bud off from their bases a thick plexus of ganglion cells and fibres. The floor of this pit may be termed the **apical plate,** and compared with the apical plate of the Tornaria larva (Fig. 389).

As the critical period of metamorphosis approaches, rudiments of spines make their appearance on the three first-formed basal plates. These differ from the spines already described, which are formed from the floor of the amniotic cavity, in being quadrangular in section with a crown of diverging points, and in being devoid of the basal collar of muscular and nervous tissue so characteristic of the adult Echinoid spine.

Other calcareous plates, the **terminals**, corresponding to the **ocular plates** of the adult, are developed on the left side of the larva outside the amniotic cavity.

At a time varying between forty-five and sixty days from the time of fertilization, metamorphosis occurs, and the larva changes into a young urchin which we may term an **imago**. The roof of the amniotic cavity becomes absorbed in the centre, and the primary azygous tube feet are protruded. In the centre of the disc of each there is a sense-organ consisting of a conical prominence of elongated ectoderm cells. At the base of each a pair of buds may be detected (*t.f*, Fig. 390), which are the rudiments of the first pair of tube feet. The peripheral portions of the amniotic roof shrink back, so that the terminal plates which they contain come to lie externally to the tube feet.

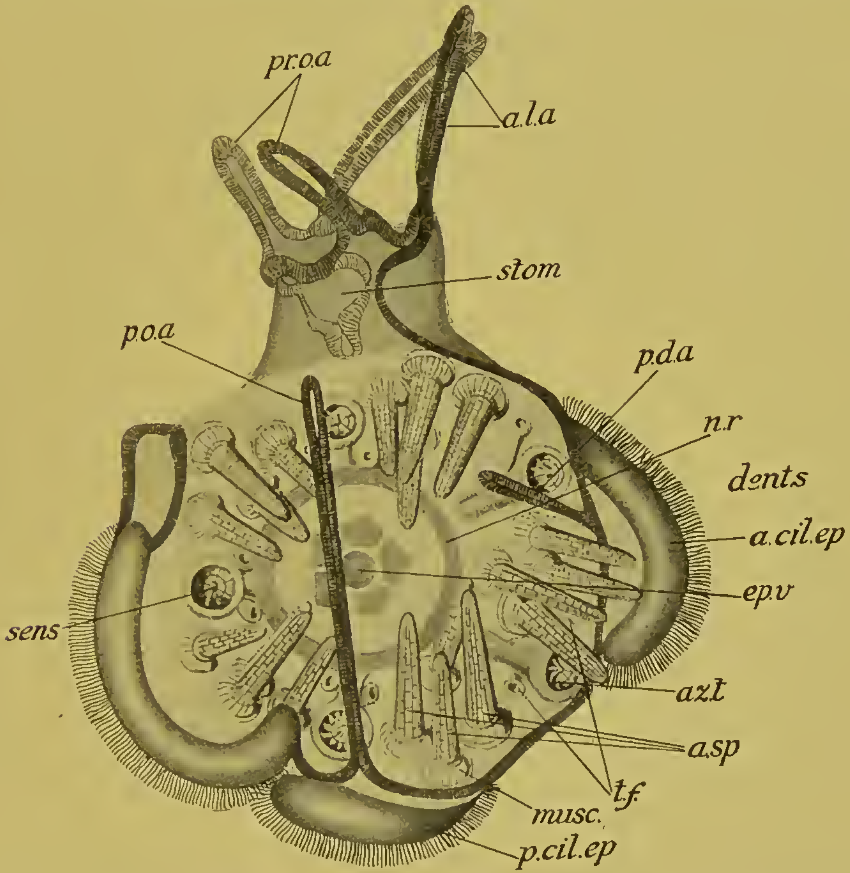

FIG. 390.—Echinopluteus larva of *Echinus esculentus* about fifty days old, just about to metamorphose, viewed from the left side. (Original.)

Letters as in Figs. 386 and 387. In addition, *az.t*, azygous tentacle, primary tube feet; *dent.s*, dental sac; *ep.v*, epineural veil, covering the position of the adult mouth; *musc*, muscles of adult spines; *n.r*, adult nerve ring; *sens*, sense-organ at apex of primary tube feet; *t.f*, rudiments of first pair of tube feet.

The larva, rendered heavy by the growth of calcareous matter, sinks to the bottom, and its tube feet adhere to the substratum. The larval arms are rapidly absorbed, beginning with the postero-dorsal on the left side. The ectodermal covering of these arms flows back towards their bases; their supporting rods protrude as naked spines but are soon broken off. The larval stomodaeum becomes disconnected with the stomach and forms a shallow pit; the oral lobe, with the stumps of prae-oral and antero-lateral arms in which this pit lies, persists for a short time but is soon absorbed. The larval anus disappears, but the larval intestine persists.

At the same time there is a great increase in the mesenchyme, which is budded off principally from the left posterior coelom, but also from the right, and a corresponding diminution in the still fluid jelly of the blastocoele. The direction right to left in the Echinopluteus becomes the direction oral-aboral in the young sea-urchin, but this axis is greatly diminished in length as the metamorphosis proceeds, and thus diminution in length must be due to the expulsion of fluid.

A most remarkable change comes over the staining properties of the ground-substance of the blastocoele at the time of metamorphosis. During the whole of the larval period it is exceedingly difficult to stain, but at the time of metamorphosis that portion of this substance immediately surrounding the stomach becomes capable of taking up stain with avidity. This must be due to the exudation of some proteid substance by the cells of the stomach. This exudation is the forerunner of an exudation which goes on all through life, and constitutes this portion of the blastocoele into the **blood system** of the adult. It is in reality to be compared with the lymphatic vessels ensheathing the human intestine, and, in the adult, the connective tissue thus modified, instead of forming a continuous mantle round the stomach, forms a network especially concentrated along two lines forming the so-called **dorsal** and **ventral vessels.**

The imago thus launched on its career is very different from the fully grown adult. It has at first neither open mouth nor anus, for the adult mouth, although already formed, is hidden beneath the epineural veil. The gut forms a simple coil, identical with the curvature of the larval gut. The five azygous tube feet, which terminate the radial canals, are the only organs of locomotion, and the canals extend horizontally outwards; there is no sign of that meridional course so characteristic of the adult urchin. The slightly convex dorsal surface is the rudiment of the insignificant adult **periproct,** but as yet it is greater in extent than the ventral ambulatory surface. In fact, if the long primary tube feet were supported by outgrowths of the body we should have a young Asteroid before us.

There is little doubt that this post-larval stage represents a former Asteroid ancestor. The changes which convert this flat discoid organism into the globular adult are almost of as great importance as those which convert the Echinopluteus into the urchin; they take place very gradually as growth proceeds. The mouth becomes open

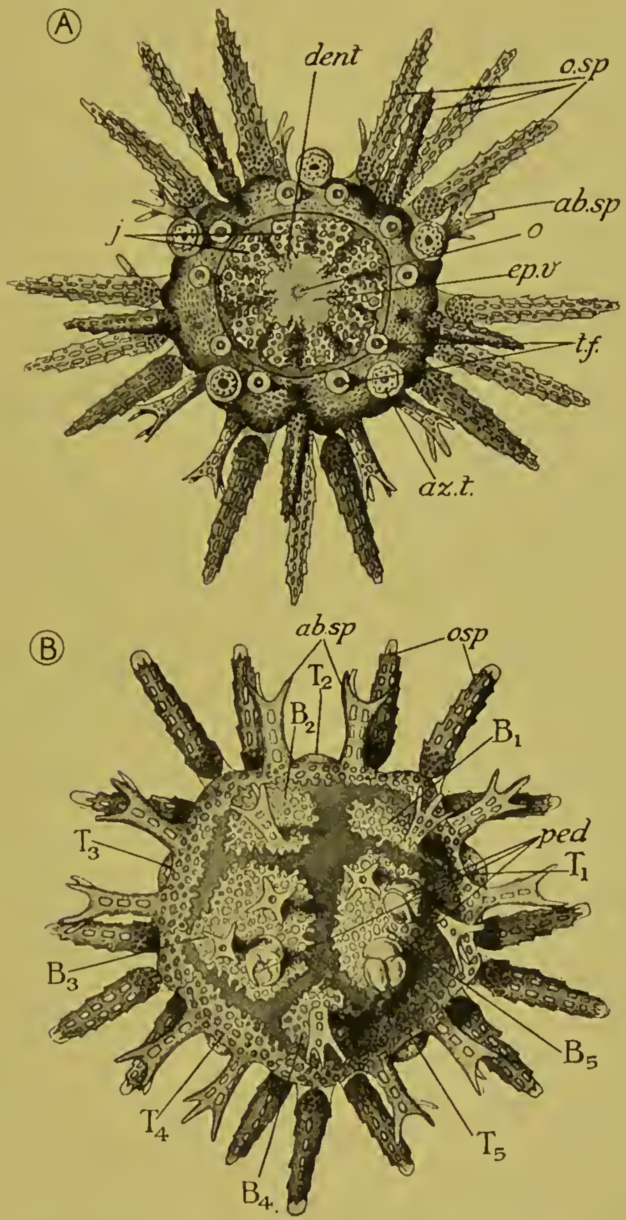

FIG. 391.—Oral and aboral views of young sea-urchins immediately after the metamorphosis. (After von Übich.)

A, oral view of young imago of *Echinus microtuberculatus*. B, aboral view of young imago of *Strongylocentrotus lividus*. B_1-B_5, the five basal (genital) plates. *ab.sp*, aboral spines of imago ; *az.t*, azygous (primary) tentacle ; *dent*, rudiment of tooth ; *ep.v*, epineural veil ; *j*, rudiment of jaw ; *o*, position of adult mouth ; *o.sp*, oral spines of imago ; *ped*, pedicellaria ; T_1-T_5, the five terminal (ocular) plates ; *t.f*, paired tube feet.

by the shrivelling of the epineural veil, and if the little animal be kept in salt water which is aerated by forcing air into it, *and in* *which one or more stones covered with the calcareous tubes of Serpulids are placed,* it seems to find food and conditions congenial to it, and it rapidly grows in size. The paired tube feet enlarge and become functional; the oral surface grows more quickly than the aboral one, and the primary tube feet are thus forced upward. As new pairs of tube feet are developed in each radius between the first-formed pair and the primary azygous one, the latter becomes of less importance, ceases to grow, and becomes enclosed in grooves in the terminal plates, which have greatly increased in size.

Alternating with the five terminals are five plates, the so-called basals, one of which surrounds the water-pore, and which had already appeared in the late larva. On the ventral surface, in the substance of the epineural veil, ten plates appear, two in each interradius. Beneath each pair the tip of the incipient **tooth** can be seen (von Übich, 1913). The teeth probably correspond to the mouth angle plates of Ophiuroids and Asteroids; the paired plates, which are the rudiments of the **alveoli** or **jaws**, to the first pair of adambulacral plates in the arms of Asteroids and Ophiuroids (*j*, Fig. 391, A). These correspond to the so-called ambulacral plates of the test of *Echinus*, which are external to the nerve cord, not internal to it as are the true ambulacrals of Asteroidea. The internal position of the first pair of "adambulacrals," which form the jaws, is correlated with the invagination of the ectoderm round the mouth, in Echinoidea, to form an adult stomodaeum.

When once the epineural veil is torn and the mouth becomes functional, the teeth grow quickly and project externally as long pointed spines, presenting quite a different appearance to their retired situation when adult. Numerous spines are added, but these are all similar to those which were formed from the floor of the amniotic cavity. The quadrangular immovable spines are confined to the aboral surface, which is rapidly diminishing in relative size. New pedicellariae are formed, and especially conspicuous are five "**sphaeridia**," one in each radius. These sphaeridia are short-spherical spines in which the calcareous skeleton is a clear solid mass.

When the little urchin has doubled its diameter the anus is formed. As the larval intestine has persisted as a blind pouch, lying in the mesentery, separating left from right posterior coelom (now oral from aboral), and as the new anus is formed by a growth of the intestine along this mesentery, it is obvious that the new anus is formed about where the old one became closed.

The larval stomach is transformed into the first or lower coil of the adult gut; the second or **recurrent coil** is gradually formed by the increase in length of the larval intestine; since the mouth and anus are fixed points, this increase in length can only be relieved by the bending back of the gut into the second coil (Fig. 392).

The **genital rachis** and the protecting **aboral sinus** are formed just as in Asteroidea and Ophiuroidea. From the rachis five interradial strands grow out which form the beginnings of the genital organs. The rudiment of the genital stolon, as we have already noted, is formed as a bud from the wall of the left posterior coelom, just as it is formed in Asteroidea. As it grows downwards in the wall of the axial sinus it becomes more extensive than in Asteroidea, since it does not merely fill a fold in its wall, but almost completely surrounds the cavity. The madreporic vesicle also grows downwards alongside the axial sinus. As in Asteroidea, the originally single madreporic pore becomes divided up by folds into the numerous pores of the adult.

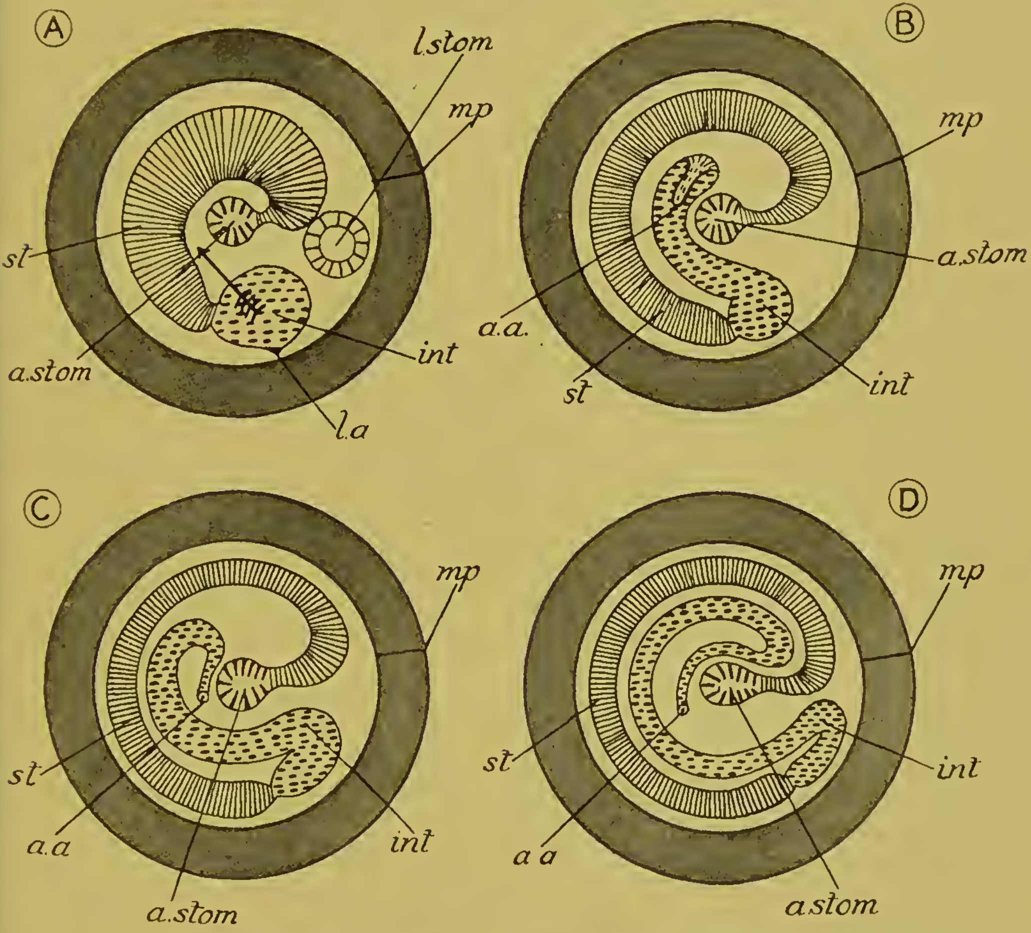

FIG. 392.—A series of diagrams showing the changes which the gut undergoes in *Echinus esculentus* after metamorphosis. The diagrams represent horizontal sections through the young imago.

A, the condition just after metamorphosis. B, the condition at the time of formation of the adult anus. C, the beginning of the formation of the recurrent coil of the intestine. D, the condition in the adult. *a.a*, adult anus; *a.stom*, adult stomodaeum; *int*, intestine; *l.a*, larval anus; *l.stom*, larval stomodaeum; *m.p*, position of madreporite; *st*, stomach.

When we compare the life-history just described with the life-histories of the two Echinoderm types previously described, we see that the Echinopluteus and Ophiopluteus resemble one another in the absence of a fixed stage, and in the consequent retention of the larval locomotor organs till the tube feet become functional. But the Echinopluteus resembles the Asteroid larva, and differs from the Ophiuroid larva in forming the adult mouth on the left side. Now we saw that in all three larvae the larval stomodaeum shallows out and disappears, but that in the Ophiuroid, before this happens, the primary tube feet protrude into the stomodaeum. We may thus interpret the amniotic cavity of the Echinopluteus, into which the tube feet protrude, as a portion of the stomodaeum which is formed separately from the rest; that is, instead of all the stomodaeum being formed at once and then being twisted to the left, it is formed in two pieces, one of which persists during larval life and then disappears, whilst the other shelters the growing tube feet and then shallows out to form the ectoderm covering the oral surface of the adult.

ECHINOCYAMUS AND MELLITA

The development of *Echinocyamus pusillus* (Theel, 1892) and of *Mellita testudinata* resembles that of *Echinus esculentus* in all essential points. The calcareous rods supporting the prae-oral arms are, however, in the form of a lattice-work, consisting of three parallel rods connected by cross pieces, and ciliated epaulettes are not formed.

In *Mellita*, according to Grave (1902), there is constantly present, in addition to the normal pore-canal, a right pore-canal opening into the right anterior coelom, and the two pore-canals unite to open by a median madreporic pore.

The larva of *Echinocardium cordatum*, the development of which we ourselves have studied (1914), develops no ciliated epaulettes, but from the second day of development a club-shaped **aboral spike** is developed, supported by a lattice-work skeleton consisting of three diverging rods connected by cross pieces. This bears a crest of ciliated epithelium. The skeleton of this club is based on transverse braces connecting up the aboral ends of the recurrent and body rods. The larva also develops horizontally extended **postero-lateral arms**, which are supported by branches of the skeleton of the aboral spike, and also a pair of **antero-dorsal arms** supported by branches from the rods supporting the prae-oral arms.

As metamorphosis approaches the aboral spike is absorbed. A very large number of adult spines are formed before metamorphosis, and the young urchin presents many points of resemblance to a regular urchin. Thus the mouth is in the centre of the aboral surface, and is surrounded by five pointed spines which are very possibly homologous with the teeth of regular urchins.

EXPERIMENTAL EMBRYOLOGY. ECHINOIDEA

We must now give a brief account of the principal results obtained by the experiments performed on the eggs of Echinoidea.

These fall into two categories: (1) Experiments on fertilization; (2) Experiments with the developing embryo and larva.

1. Seeliger (1894) has proved that if an egg be broken into two parts, one of which possesses the nucleus and one of which does not, both fragments can be fertilized, and both will develop into apparently normal larvae. Loeb (1910) has proved that if the alkalinity of the sea-water be slightly increased by the addition of from 1 to 2 per cent of $\frac{n}{10}$ NaHO, it is possible to fertilize the eggs of some species of Echinoidea with the sperm of Asteroidea, Ophiuroidea, and Crinoidea. Godlewski (1906) has even fertilized the enucleated fragments of Echinoid eggs with Crinoid sperm, and has obtained gastrulae. Finally, Kupelwieser (1909) and Loeb have succeeded in fertilizing Echinoid eggs with the sperm of Mollusca (*Chlorostoma and Mytilus*), and in obtaining larvae which resemble closely the normal four-armed Echinopluteus.

In some cases, as Kupelwieser has shown (1909), the chromosomes of the spermatozoon do not enter the first spindle of division which is formed in the egg; so that the action of the spermatozoon must be regarded as stimulating the egg to parthenogenetic development, but not as conveying its own hereditary qualities; and in these cases the resulting larvae show purely maternal characters. But this may be the case even where a genuine fertilization appears to take place, and the chromosomes of the spermatozoon enter the first spindle, as Godlewski showed is the case when the uninjured eggs of Echinoidea are fertilized with Crinoid sperm.

Still more startling is it to find that this may also be the case, as Shearer, Fuchs, and De Morgan (1912) have shown, with the hybrids of *Echinus esculentus* and *E. miliaris*, two species which are easily crossed. Whichever way the hybrid is made it may exhibit purely maternal characters, and these hybrids have been reared through the metamorphosis. These authors have also shown that the character of the hybrid obtained by fertilizing the eggs of *Echinus miliaris* with the sperm of *Echinus esculentus*, varies from year to year; in one year it was purely maternal in character, in the next of a type intermediate in character between the larvae of the two parent species. Even the hybrid obtained by crossing forms belonging to different orders of Echinoidea is not always maternal in character, as we ourselves showed by crossing *Echinus esculentus* and *Echinocardium cordatum*. The hybrids between these two species only live eight days, nevertheless, although the two parents are very widely separated in systematic position, the hybrids show unmistakable paternal features as well as maternal ones.

The question now arises whether, if parthenogenetic development can be initiated in the eggs of Echinoidea by substances contained in the sperm of Mollusca, which do not convey any hereditary qualities, it could not also be initiated by dead solutions of an organic or inorganic character. This question has been answered in the affirmative. Morgan was the first to discover that the eggs of Echinoidea, if treated with hypertonic sea-water, that is, water in which the normal proportion of chlorides is increased, will occasionally undergo irregular segmentation and develop into larvae with feebly developed arms, which lie on the bottom.

Loeb (1910) confirmed this result, but he showed that if the eggs are first treated with an organic acid, like butyric or valerianic acid, for a minute or two, they will form a membrane exactly like the vitelline membrane; then regular segmentation will commence and lead to the formation of normal larvae which swim at the surface. This occurs in but a very small percentage of cases, while the rest perish by a process of cytolysis whereby the blastomeres break up into globules. If the eggs, after being treated with the organic acid, are rinsed in clean sea-water and then transferred to hypertonic solution, and after a time to clean sea-water, all will develop into normal larvae. It is to be noted that the time differs in the case of the eggs of every female examined, and has to be experimentally determined in each case.

From such experiments Loeb draws the conclusion that the formation of a vitelline membrane is an important step in normal development: that this membrane is due to the beginning of a process of cytolysis initiated by the spermatozoon, since careful examination shows that the first stage in its development is the formation of a layer of minute globules which flow together; but that the spermatozoon also contains a material which checks this cytolysis when it has gone far enough, and that both these actions are independent of what may be called the hereditary influence of the spermatozoon.

2. We now pass to experiments made on embryos and larvae. Driesch began his investigations (1892) by taking normally fertilized eggs of sea-urchin, waiting until they had divided into two or four blastomeres, and then separating them by violent shaking. In this way he was able to show that even one cell of the 8-blastomere stage will produce a perfect larva, so that in this species the division into cells is not, as Roux supposed, a division of the germ into areas destined to form special organs.

He further showed that when one of the first two blastomeres was separated from the other, the isolated blastomere segmented as if it still formed half of the egg. Thus a hemispherical cup of cells was formed, and the edges of this cup were then drawn together by movements of the cells composing it. In this way a blastula was formed with half the normal number of cells, which nevertheless developed into a perfect larva.

He was also able to induce the eight cells of the 8-cell stage to arrange themselves in one plane, and this was accomplished by freeing the eggs from their fertilization membrane by means of shaking them a few minutes after fertilization, and by subjecting them to pressure. If then the pressure were removed the eight cells changed into the 16-cell stage, consisting of two tiers of eight; this 16-cell stage then became a normal blastula and subsequently developed into a normal larva.

It followed that nuclei, which normally would have been situated at one pole, would now be found at the sides of the larva, and hence he drew the conclusion that the nuclei are indifferent structures and may be exchanged for one another without altering the course of development.

The same result can be obtained by subjecting the developing egg to a moderate degree of heat, viz. a temperature of about 25°; the four lower cells of the normal 8-cell stage will arrange themselves in one plane with the cells of the upper tier.

When the eggs were freed from their membranes by shaking and then exposed to water freed from calcium, they segmented normally, but the segments fell apart. This peculiar effect of sea-water devoid of calcium has been alluded to in previous chapters. It was first demonstrated by Herbst (1900). When this happened in the 16-cell stage, when micromeres, mesomeres, and macromeres had been differentiated, it was comparatively easy to recognize the fragments, which usually consisted of from two or four cells, by the size of their component cells (1900). It was found that such fragments, when derived from the micromeric pole, generally died, but that when they developed they grew into perfect larvae; whereas those from the anti-micromeric or animal pole, on the other hand, generally lived and developed into clear, free-swimming blastulae, but could develop no further.

From this circumstance Driesch drew the conclusion that the substances which are necessary for the formation of the mesenchyme and gut are, in the 16-cell stage, chiefly centred at the lower pole of the egg; hence when cells from the upper pole are taken they can never form these organs.

These conclusions were confirmed by cutting the blastulae of *Sphaerechinus* in pieces by a pair of fine scissors, as was done with the gastrulae of *Asterias*. In early stages the fragments healed up and formed perfect larvae, but, once the primary mesenchyme was formed, then fragments from the vegetative half could form gut and mesenchyme but not the long apical tuft of cilia, while fragments from the animal half formed the apical tuft of cilia but never could form gut and mesenchyme.

Sometimes, by the action of the calcium-free water, the two first blastomeres were incompletely separated; then there resulted a flattened blastula which was oval, not circular in section. The long axis of the ellipse was obviously the line joining the two nearly separated blastomeres. When the first signs of bilateral symmetry, which in this case are the calcareous stars, appeared, they were situated at opposite sides of the *short axis* of the ellipse. It is thus proved that the first cleavage furrow is transverse to the median plane of symmetry, and that in normal development the first two blastomeres produced from the egg are anterior and posterior, not right and left.

When the gastrulae of *Sphaerechinus* are cut up, even if the archenteron has already differentiated itself into oesophagus, stomach, and rectum, if the fragment contains a part of the gut this will differentiate itself again into oesophagus, stomach, and rectum on a smaller scale.

This result Driesch holds to be incompatible with any physical or chemical explanation, but to render necessary the hypothesis of an indwelling non-material entity—the **entelechy**, which directs the material at its disposal to purposeful ends. Driesch will not speak of organ-forming substances, but of conditions, and he claims that when the cytoplasm of the egg has been regulated to a certain extent by the entelechy, in a certain direction, it becomes "stiffer" and more incapable of "regulation" in a different direction.

But there is the most indubitable evidence that, whether we can form a mental picture of them or not, such things as organ-forming substances do exist; for instance, when the developing tail of a lizard, after an injury, can be made to give rise to two tails merely by indenting the growing rudiment, what other explanation can be given? Can the indwelling entelechy be forced to alter its whole method of action by such an external influence? Even Driesch's *pons asinorum* can be got over, as will be shown below.

When the blastulae of sea-urchins, instead of the segmentary eggs, are exposed to heat, the gut develops as an external evagination instead of an invagination. This external appendage can be cut off, and in this way an anenterous larva is formed. Such a larva can develop the first four pluteus arms and the stomodaeum. In this way it can be proved that the formation of the stomodaeum is independent of the gut.

Herbst (1893, 1895, 1896) took up the task of testing the action of different chemicals on the course of the development. He made solutions of different salts in distilled water, of approximately the same strength as is constituted by the total salt in sea-water. Small quantities of these solutions were then added to the sea-water in which the eggs to be experimented with were placed. He found that the results obtained depended chiefly on the basic radicle in the salt, and that they were inversely proportional to the molecular weight of the salt used; or, in other words, directly proportional to the number of molecules. Most of the bases tried produced a larva devoid of calcareous centres. Such larvae never produced larval arms.

Herbst's most startling result was obtained by the use of the salts of lithium. To obtain these results the eggs must remain in the mixture of sea-water and lithium solution from the time they are fertilized till the blastula stage is reached. If removed sooner the lithium salt produces the same result as the other salts used, and eggs submitted to the action of the solution after sixteen cells have been formed do not yield the typical result now to be described.

If eggs are left in the lithium mixture from the time they are fertilized until the blastula stage is reached, then they develop into motionless larvae which show by their clear protoplasm that they are healthy. These larvae are divided by a constriction into two sections, one of which shows by the nature of its cells that it corresponds to an inverted gut, and the other corresponds to the ectodermic skin of the gastrula. The effect, then, of the lithium would be to alter the conditions of pressure in the blastocoele so that the gut develops outwards and not inwards.

If, as we have seen reason to believe, the process of gut formation is analysable into two factors, viz. *cell multiplication* and *inwardly directed cytotaxis*, then the lithium has reversed the direction of the cytotaxis. But that is not all. The longer the eggs lie in the mixture of sea-water and lithium solution, and the more of the lithium solution used, *the bigger is the portion of the larva which corresponds to the gut.* This can go so far that practically the whole of the larva becomes gut, the ectoderm being represented by a little knob of cells at one end of the vesicle.

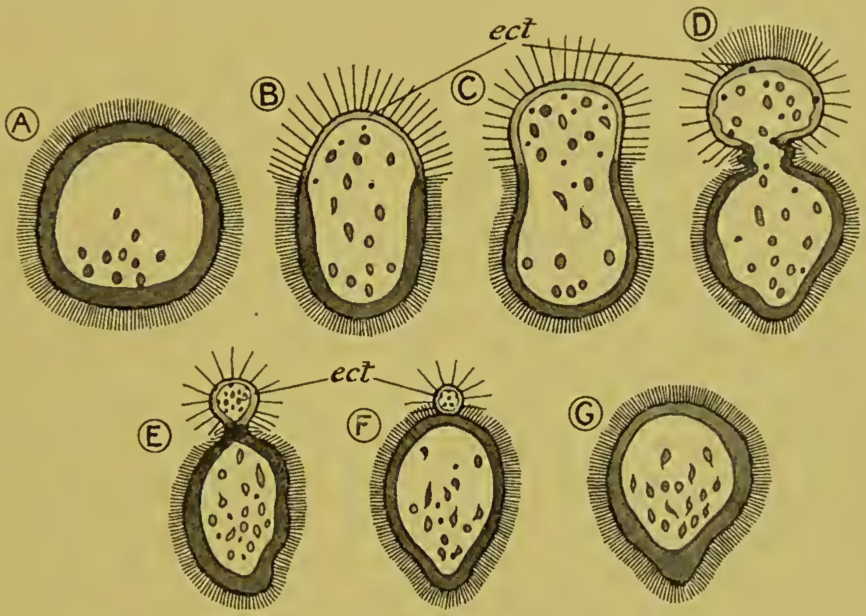

Fig. 393.—Types of lithium-larva. (After Herbst.)

A, blastula stage of lithium-larva of *Sphaerechinus granularis*; ectoderm and endoderm are not yet differentiated. B, later stage in the development of the same larva; the ectoderm carries the long cilia. C, still later stage in the development of the same larva; the ectoderm is beginning to be grooved off from the endoderm. D, still later stage; the groove is deep, and the endodermic portion exhibits a secondary division into a small upper part representing the intestine (?), and a lower part representing the stomach of a normal larva. E, F, G, lithium-larvae of *Sphaerechinus granularis*, in which the lithium has acted more intensely, in E the ectodermic portion is very small, in F it is reduced to a mere knob, in G it has disappeared. *ect*, ectodermic part of larva.

Herbst suggests that when the blastula stage is reached the physiological separation of ectoderm-forming and endoderm-forming substances is hurried on. He supposes that, in a normal blastula, the amount of endoderm-forming substance increases as we proceed from the animal to the vegetable pole. When the endoderm-forming substance accumulates, absorptive power is increased and more lithium is taken in. The effect of this is to increase still further the amount of endodermic substance and inhibit the formation of ectodermic substance, and so more and more of the blastula wall is transformed into endoderm.

When larvae in which there is still some ectoderm are transferred to sea-water, they acquire the power of ciliary movement, and develop an abnormally large number of calcareous spicules which are arranged in a circle, and a correspondingly large number of arms is developed; so that the formation of an arm is dependent on the stimulus afforded by the presence of a spicule.

We ourselves (1911) have found that in the larvae of both *Echinus miliaris* and of *Echinus esculentus* a right hydrocoele is occasionally developed. In such cases a right amniotic cavity may be developed, on the floor of which typical pointed spines may arise. Further, from the right posterior coelom a series of dental sacs or perihaemal rudiments may be formed, and finally a second "adult" oesophagus and mouth. This shows that neither the normal formation of an amniotic invagination from the ectoderm of the left side, nor of dental sacs from the left posterior coelom, nor of an adult oesophagus from the left side of the stomach, is due to the pre-existence of an invisible rudiment of the structure to be found in the layer of cells out of which it is formed; on the contrary it is clear all three developmental processes mentioned must be due to an influence from the hydrocoele acting on the indifferent sheets of tissue constituted by these layers, *i.e.* on ectoderm, coelomic wall, and stomach endoderm respectively. Such influences are termed **formative stimuli.**

Hence we may make to ourselves the following provisional picture of Echinoid development. Very early, before cell division has occurred, there are constituted in the cytoplasm, by the influence of the nucleus, definite substances which can cause the formation of the primary organs. The formation of the substances cannot be attributed to the daughters of the primary nucleus, because then it would be impossible to disarrange these daughter nuclei and yet obtain a typical result.

These substances are at first uniformly distributed throughout the egg; as segmentation goes on they become segregated from one another, *and when this segregation is complete the formation of the layers, that is, of the primary organs, is begun.* When once these layers are formed they in turn produce substances which act on each other and cause progressive differentiation. These substances can be increased or decreased by the action of certain salts. So long as a fragment of an egg has a certain minimal proportion of an organ-forming substance, the organ will be formed in typical development.

Driesch's *pons asinorum* regarding the formation of a gut of small size, differentiated into its typical regions, out of a fragment of a gut of larger size, can be surmounted, as Zur Strassen showed, by the assumption of "formative stimuli" proceeding from the cut ends of the gut fragment, and determining by their interaction the formation of constrictions in what was, at first, a uniform tube.

The great difference, then, between the eggs of Echinoidea and those of Nemertinea, Ctenophora, Annelida, and Mollusca is that in the latter eggs the organ-forming substances are separated from one another at a far earlier date in development; that, in fact, in these groups the segmentation of the egg is not merely the multiplication of nuclei, as it is in Echinoidea, but is already incipient organogeny.

HOLOTHUROIDEA

Our knowledge of the development of Holothuroidea constitutes the least satisfactory part of our knowledge of the embryology of Echinodermata. A complete series of stages of the external form is only known in two or three life-histories, viz. those of *Synapta digitata*, *Synapta vivipara*, and *Cucumaria planci*. In only one of these cases is a larva formed which leads a free-swimming life for a considerable time, and which can be compared to the Bipinnaria, Ophiopluteus, and Echinopluteus larvae which have been described.

The eggs of *Cucumaria planci*, and so far as we know of other species of *Cucumaria* as well, are yolky, and undergo a shortened development, in which the larva, although a free-swimming organism, takes no food but depends for its nourishment on yolk grains stored in its cells, like the larva of *Solaster endeca*.

The young of *Synapta vivipara* undergo the whole of their development within the body-cavity of the mother, and yet this is the form which has been most carefully investigated and in which modern methods have been most conscientiously applied. We owe our knowledge of the development of this form to Lyman Clark (1898), and his results seem to show that in most respects, so far at least as the formation of internal organs goes, the development of this form agrees with that of *Synapta digitata*. This latter we shall select as type, but we must warn the reader that our knowledge of its development is very scanty compared to our knowledge of the development of *Asterias rubens*, of *Ophiothrix fragilis*, or of *Echinus esculentus*.

SYNAPTA DIGITATA

Our account of the early stages we owe to Selenka, who alone has artificially fertilized the eggs of this form (1893). Semon has given a highly defective account of its development and of its metamorphosis, based on larvae caught in the Plankton, and has made this account the ground for a most fantastic and improbable theory of the phylogeny of Echinoderms (1898). Bury (1889 and 1896) devotes portions of two most valuable papers to the consideration of the development of *Synapta*, and corrects many of Semon's misstatements. It is the more to be regretted that *Synapta* has to be chosen as type, because in its adult anatomy it is one of the most aberrant members of the class Holothuroidea.

The early development of *Holothuria tubulosa* has been described by Selenka (1876); this species gives rise to a larva like that of *Synapta*, but the adult is one of the least modified of Holothuroidea. Selenka did not keep his larvae alive more than four or five days. It is much to be desired that the development of this form should be studied again. By using pure cultures of *Nitschia* as food the larvae of *Cucumaria saxicola* have been reared throughout the whole of their development at Plymouth.

Selenka only found one ripe female *Synapta* amongst all the hundreds which were brought to him by the collectors of the Naples Station. It is probable that this was partly due to the season of year at which he visited Naples, and partly to the fact that Holothuroids are not so easily obtained in large numbers as are Asteroids, Ophiuroids, and Echinoids, at any rate on British shores. *Synapta* leads a burrowing life in sand and gravel, and is frequently not reached by the dredge; the most hopeful place to find it would be by search during extreme low tides in sheltered inlets like the Clyde.

The other genera, such as *Holothuria*, *Cucumaria*, etc., seem to hide in crevices amongst stones, and only occasional stragglers are caught by the dredge. Selenka (1876) secured large numbers of *Holothuria* by a contrivance resembling a lobster trap, viz. a box with the lid fastened down but with a small hole in the top, which he sunk in the sea.

The segmentation of the egg of *Synapta*, as described by Selenka, is the most regular as yet observed in the animal kingdom. The egg divides into two and then four oval segments, and these divide into two tiers of four each. At the next cleavage each cell divides by a radial furrow into two daughters lying side by side; in this way we get two tiers of eight cells each.

Already in the 4-cell stage the first trace of the **blastocoele** made its appearance as a separation of the blastomeres from one another in the centre of the egg; in the 32-cell stage, which consists of four rings of eight cells each, these cells surround a wide space open above and below (Fig. 394). In the following cleavage each cell divides by a longitudinal furrow so that the number of cells in each tier is doubled. The rings nearest the equator continue to divide by alternate radial and meridional furrows, but in the upper and lower tiers some of the cells migrate pole-wards and form here rings of smaller diameter, so that the open spaces at the poles are covered in and a closed **blastula** results.

When 512 cells have been formed, that is at the conclusion of the ninth cleavage, each develops a cilium and the blastula begins to rotate within the egg-membrane. The blastula elongates, and a slight thickening occurs at one pole since the cells in this region become columnar. This thickening we may regard as a vestigial **apical**

plate (*ap*, Fig. 395). At the opposite pole the invagination which is to form the archenteron appears. This is of very small extent in comparison with the length of the blastula; it does not, at the period of its greatest extension, equal in length half the total length of the embryo.

When the development has reached this point the embryo escapes from the egg-capsule and begins to lead a free-swimming life. In *Synapta*, therefore, the larval life commences with the **gastrula** stage, not with the blastula as in the other groups studied.

The mesenchyme is formed only after the gastrula stage has been reached. It originates, according to Selenka, in two cells given off from the apex of the archenteron which multiply by division. This is unlikely, it is more probable that numerous cells are given off.

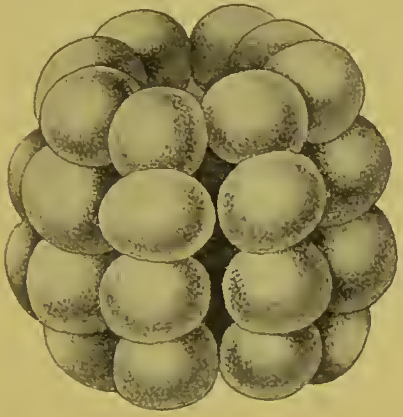

Fig. 394.—The 32-cell stage in the segmentation of the egg of *Synapta digitata* viewed from the side. (After Selenka.)

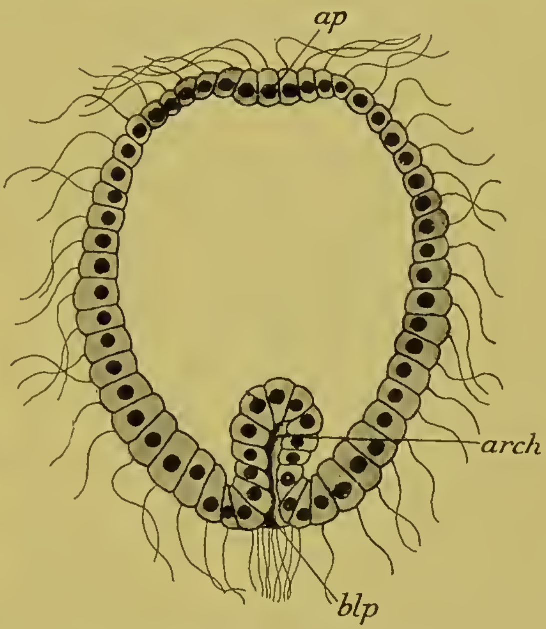

Fig. 395.—The free-swimming gastrula of *Synapta digitata*. (After Selenka.) *ap*, apical thickening; *arch*, archenteron; *blp*, blastopore.

In the late origin of the mesenchyme the *Synapta* larva resembles the Asteroid, and the reason for this may be the same in both cases, namely, the absence of a larval skeleton, to the formation of which is devoted the primary mesenchyme of Ophiuroids and Echinoids, which is given off in the blastula stage.

The tip of the archenteron bends at right angles and grows towards the dorsal side of the larva; it fuses there with the ectoderm, and a perforation of the fused layers is effected so that the lumen of the archenteron communicates with the exterior. This perforation is the **madreporic pore** (*mp*, Fig. 396). After it is formed the horizontal branch of the archenteron is separated from the vertical one and becomes the **coelom**, whilst the vertical section of the archenteron is the **gut**. This latter soon becomes divided by constrictions into **oesophagus, stomach,** and **intestine.** This last division is bent forwards, so that, as in other Echinoderm larvae, the alimentary canal is bent in a curve, concave ventrally. The **stomodaeum** is formed in the usual manner on the ventral side of the larva; it joins the oesophagus after the coelom has been separated from the alimentary canal.

The general covering of cilia becomes lost as the ectoderm cells flatten out, and the cilia become restricted to ridges which form a folded **longitudinal band.** This band resembles that of the Bipinnaria larva of Asteroidea in its earlier stages of development, *i.e.* it consists of right and left longitudinal pieces and anterior and posterior cross-bars, the former of which is bent backwards over the prae-oral region of the larva as the **prae-oral loop,** and the latter of which is bent forwards in front of the anus as the **anal loop.** An **adoral band** of cilia is formed in the same way as has been described for the Bipinnaria, Ophiopluteus, and Echinopluteus.

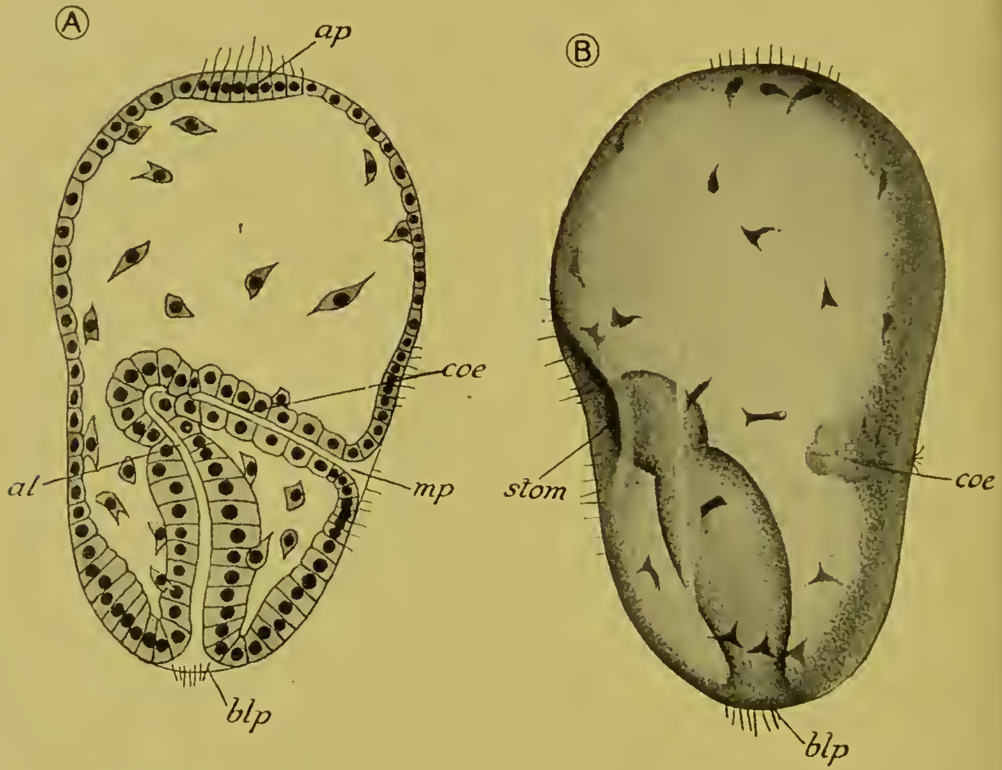

Fig. 396.—Two young larvae of *Synapta digitata* viewed from the side. (After Selenka.) A, formation of the coelom. The coelomic part of the archenteron opens to the exterior by the madreporic pore before it is separated from the gut-portion of the archenteron. B, formation of the mouth and stomodaeum. *al*, alimentary canal; *ap*, apical thickening; *blp*, blastopore; *coe*, coelomic vesicle; *mp*, madreporic pore; *stom*, stomodaeum.

The Holothuroid larva differs from the Bipinnaria in two points: first, the prae-oral loop, although almost, is never quite constricted from the rest of the band, so that the longitudinal band of cilia remains simple; and, secondly, the loop-like outgrowths of this band remain much shorter than in the Bipinnaria larva. They are usually called "processes," but as they are homologous with the arms of other types of Echinoderm larvae we shall call them by the same name.

We find **prae-oral arms** (*pr.o.a*, Fig. 398) in front of the mouth on the side of the prae-oral loop; at the sides of the anal loop **post-oral arms** (*p.o.a*, Fig. 398); and each side of the longitudinal band carries an **antero-dorsal**, an **intermediate-dorsal**, and a **postero-dorsal arm** (*a.d.a*, *int.d.a*, *pd.a*, Fig. 398). Where the anal loop originates from the longitudinal band there is a well-marked **postero-lateral arm** on each side (*pl.a*, Fig. 398). This arm is the one which, from a fancied resemblance to the human ear, suggested the name Auricularia to Johannes Müller (1850), by which name the larva is known.

AURICULARIA LARVA

From the foregoing description it is obvious how closely the Bipinnaria and Auricularia larvae resemble one another in external appearance, but the Auricularia larva differs widely from the Bipinnaria in its metamorphosis, and indeed in the internal changes which precede metamorphosis.

The coelomic vesicle is at first situated in the mid-dorsal line; it shifts to the left, and then divides into anterior and posterior portions. The posterior vesicle divides into right and left posterior coeloms, which apply themselves to the sides of the larval stomach. This change has been observed only by Metschnikoff (1869).

The anterior vesicle does not, however, as Selenka and Metschnikoff imagined, become directly converted into the hydrocoele; Bury has shown that it becomes divided into dorsal and ventral parts by a constriction (1896). The dorsal part, which communicates with the exterior by the primary madreporic pore, is the **left anterior coelom**; the ventral portion is the rudiment of the **hydrocoele**, and the connecting narrow part is the stone canal. Neither right anterior coelom nor right hydrocoele are developed.

In the Auricularia larva, then, in spite of its outward bilateral symmetry an inner asymmetry is early evident. The hydrocoele has the form of a hoop whose plane is the frontal plane of the larva, and it lies at the left side of the oesophagus. From its outer surface there arise five lobes, which are *not* the rudiments of the radial canals of the water-vascular system, but of the **primary buccal tentacles** (1_1, 2_1, 3_1, 4_1, 5_1, Fig. 398). Alternating with these lobes there appear five much smaller lobes (1, 2, 3, 4, 5, Fig. 398), which are the rudiments of the **radial water-vascular canals.**

On these facts Semon's whole hypothesis of the phylogenetic relationships of the classes of Echinodermata is built. He regards the first five lobes as equivalent to the five primary lobes of the hydrocoele in the Bipinnaria, Ophiopluteus, and Echinopluteus larvae, and therefore equivalent to the radial canals in the Asteroidea, Ophiuroidea, and Echinoidea respectively; the radial canals of the Holothuroidea, the rudiments of which are represented by the secondary lobes of the hydrocoele, would therefore, on this hypothesis, not be homologous with those of other Echinoderms.

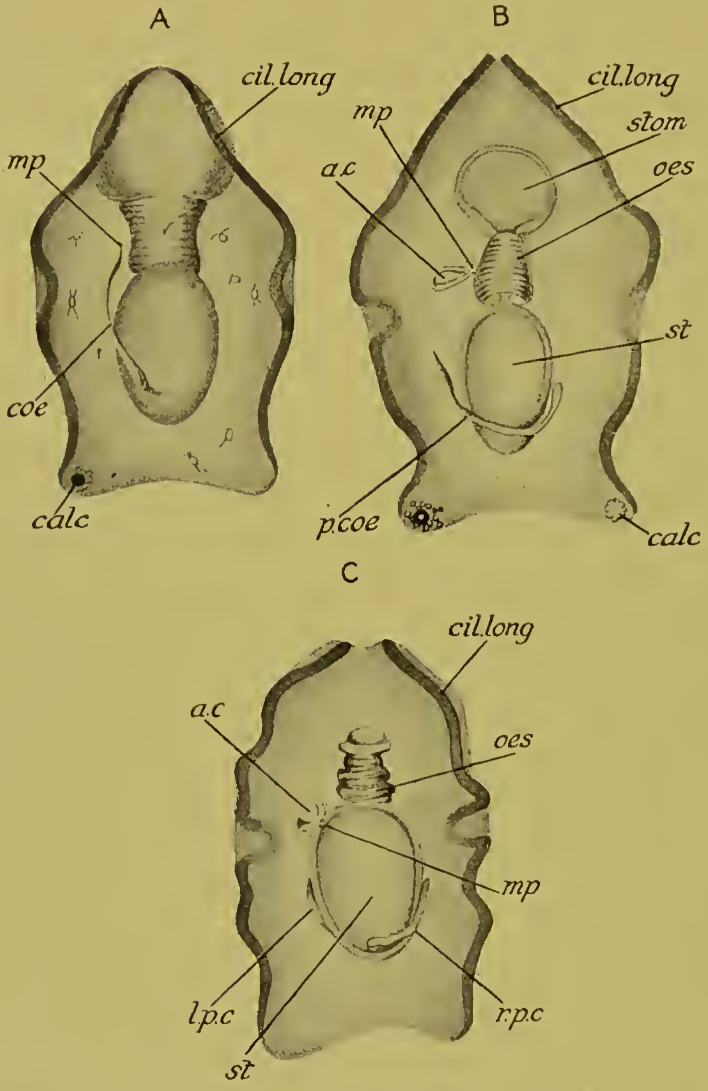

Fig. 397.—Three views of young Auricularia larvae of *Synapta digitata* viewed from the dorsal surface, showing the division of the coelom. (After Metschnikoff.)

A, the coelom still undivided, but extending far backwards. B, the coelom divided into anterior and posterior portions. C, the posterior portion of the coelom divided into right and left halves. *a.c*, anterior division of the coelom; *calc*, calcareous body; *p.coe*, posterior division of the coelom; *cil.long.* longitudinal ciliated band; *coe*, undivided coelomic sac; *l.p.c*, left posterior coelom; *mp*, madeporic pore; *oes*, oesophagus; *r.p.c*, right posterior coelom; *st*, stomach; *stom*, stomodaeum.

But the whole of this theory is shattered by our knowledge of the development of *Cucumaria planci*, first described by Selenka but worked out with care by Ludwig (1891).

In this form the first five lobes of the hydrocoele give rise to the radial canals, and the primary buccal tentacles arise from their bases. Now in *Synapta* the radial canals disappear in the adult, they are, in a word, vestigial functionless structures; but in *Cucumaria* they persist and give rise to numerous paired tube feet. Therefore the mode of their development in *Synapta* is secondary, the method in *Cucumaria* typical and primary, and this typical and primary method is the same as that which obtains in other groups of Echinoderms.

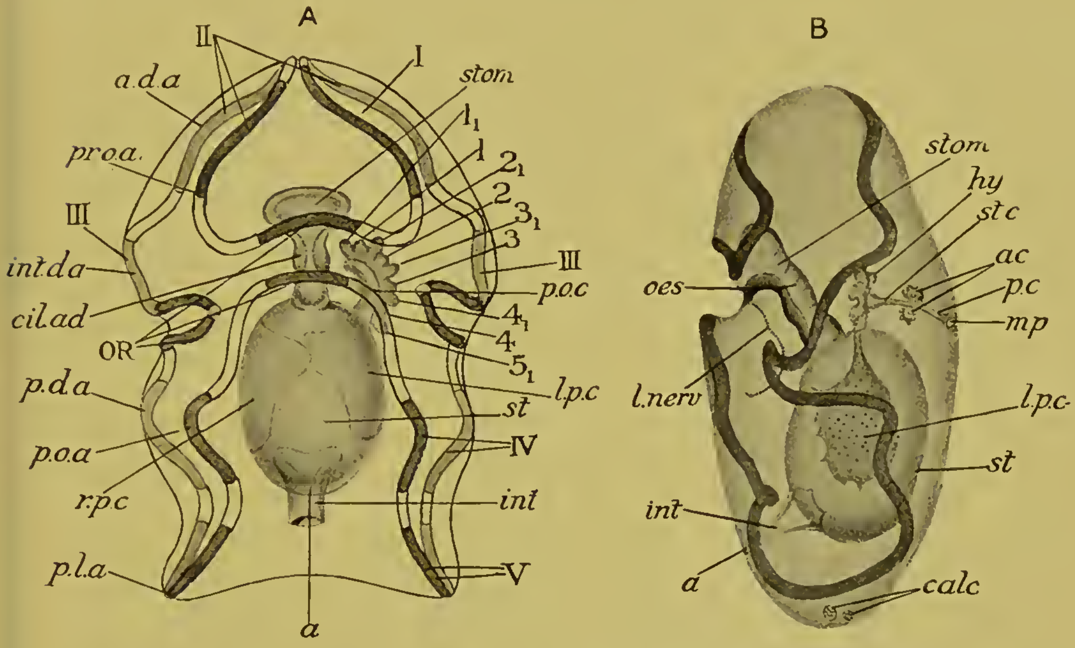

FIG. 398.—The fully developed Auricularia larva of *Synapta digitata* viewed from the ventral surface and from the side. (After Bury.)

A, Auricularia viewed from the ventral surface. The darker regions of the longitudinal ciliated band indicate the fragments into which it will break at the metamorphosis. B, Auricularia viewed from the left side. Letters as in previous figure. In addition, I-V, the fragments of the ciliated band which will give rise to the transverse ciliated bands of the pupa. OR, the fragments of the ciliated band which will give rise to the ring surrounding the mouth of the pupa. 1_1-5_1, the lobes of the hydrocoele which will give rise to the first five buccal tentacles. 1-4, lobes of the hydrocoele giving rise to radial canals. *a*, anus; *a.c*, left anterior coelom; *a.d.a*, antero-dorsal arm; *cil.ad*, adoral band of cilia; *hy*, hydrocoele; *int*, intestine; *int.d.a*, intermediate dorsal arm; *l.nerv*, larval nervous system of Semon; *p.c*, pore-canal; *p.d.a*, postero-dorsal arm; *p.l.a*, postero-lateral arm; *p.o.a*, post-oral arm; *p.o.c*, peri-oral coelom; *pr.o.a*, prae-oral arm; *st.c*, stone-canal.

The mesenchyme in *Synapta* has by this time formed a loose reticulate tissue spanning the blastocoele. In the postero-lateral process certain of the mesenchyme cells have given rise to typical calcareous structures which enable us to distinguish the larva of *Synapta digitata* from other kinds of Auricularia (*calc*, Fig. 398, B). These calcareous bodies are **wheels** consisting of a central knob, numerous spokes, and a toothed rim. The knob does not lie in the same plane as the rim, but beneath it, so that the whole might be compared to a bowl with perforated sides as well as to a wheel.

METAMORPHOSIS OF THE AURICULARIA

Signs of the metamorphosis now appear. The hydrocoele is still in the form of a vertical hoop with its concavity directed towards the right. From the anterior edge of the left posterior coelom a finger-like process grows out and extends along the hydrocoele. This is the **peri-oral coelom** (*p.o.c*, Fig. 398), homologous with the peri-oral coelom in Asteroidea and Ophiuroidea. At the same time the left and right posterior coeloms approach each other ventral to the intestine, and eventually become applied to one another in an oblique line. The left coelom extends over to the right in front of the right coelom; this extension is evidently homologous to the **right ventral horn** of the left posterior coelom in the larva of *Asterina gibbosa*. Then the longitudinal ciliated band breaks into fragments, owing, no doubt, to the fact that the neighbouring ectoderm grows more quickly than that forming the band.

The following are the pieces into which the band breaks up:—The prae-oral loop gives rise to a median and two lateral pieces (Fig. 399, A), and the anal loop gives rise to three precisely similar pieces. Each side of the longitudinal band breaks up into four pieces, three of which roughly correspond to the antero-dorsal, intermediate dorsal, and postero-dorsal larval arms. The fourth piece corresponds to the re-entrant angle between intermediate dorsal and postero-dorsal arms. Finally, each postero-lateral arm gives rise to a separate fragment of the band.

Semon maintains that even before this stage a **larval nervous system** exists (*l.nerv*, Fig. 398), consisting of two longitudinal ridges of thickened ciliated ectoderm which are independent of, and within the field embraced by, the sides of the larval ciliated band. Semon draws the conclusion that these are nervous, because in sections he finds that there are fibres at their bases; but no separate nerve-cells have ever been discovered. According to Semon, as metamorphosis proceeds, these bands become carried into the interior of the larva by an invagination which involves the mouth and form the adult nerve-ring.

Bury gives a different account of the matter, and as Semon has proved to be inaccurate in other points we follow Bury. This author points out that by inequality of the rates of growth of neighbouring parts the mouth is carried over to the left side, and the apical pole of the larva is displaced to the right. Then a ciliated ring round the mouth is formed by the juxtaposition of four pieces of the old longitudinal ciliated band: these four are the median pieces of the prae-oral and anal loops and the pieces of the sides of the band mentioned above, which correspond to the re-entrant angles between intermediate-dorsal and postero-dorsal arms on each side (Fig. 398).

Then the invagination described by Semon occurs, by which the larval mouth surrounded by the circular ring comes to lie at the bottom of a deep pit, the aperture of which nearly but not quite closes; the opening of this **atrium** is distinctly on the left side. The remaining fragments of the longitudinal ciliated band then join with each other so as to form five transversely arranged ciliated rings. Of these the fifth and most posterior is formed by the union of the fragments formed from the two postero-lateral processes. The next, *i.e.* the fourth ciliated ring, is formed by the union of four fragments, two derived from the remains of the postero-dorsal processes, two from the lateral fragments of the anal loop. The next, *i.e.* the third ciliated ring, arises from the union of the fragments produced by the intermediate dorsal processes.

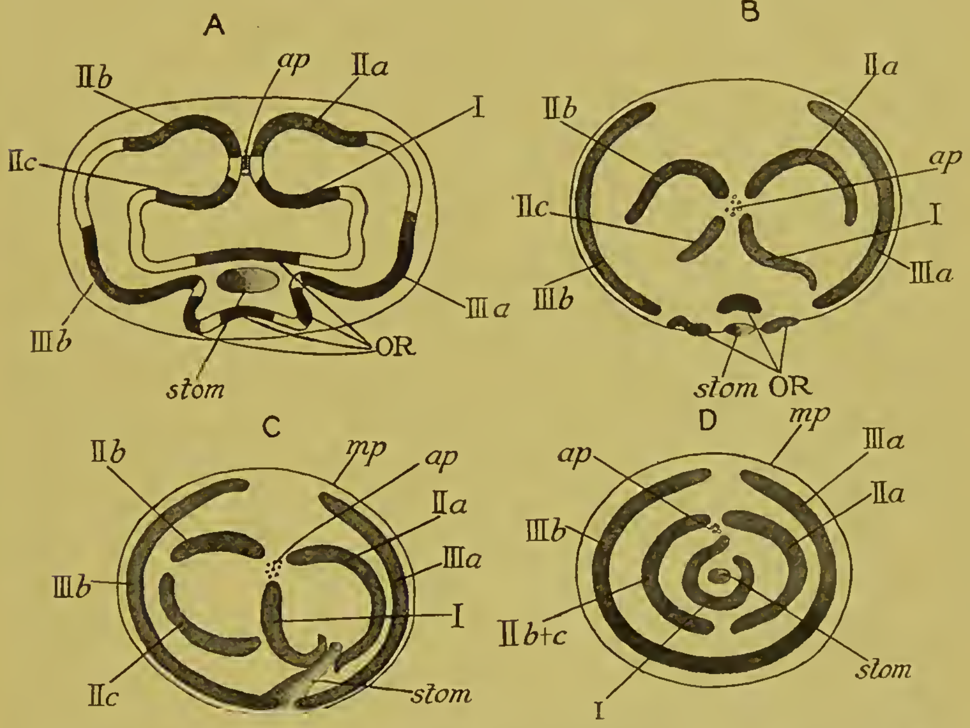

FIG. 399.—Diagrams of the anterior aspect of metamorphosing larvae of *Synapta digitata* in order to show the changes undergone by the longitudinal ciliated band. (After Bury.)

A, before metamorphosis into the pupa. B, C, during metamorphosis into the pupa. In C the mouth has moved to the left. D, after the metamorphosis into the pupa has been almost completely accomplished. I, fragment which gives rise to the first transverse ciliated band of the pupa. IIa, IIb, IIc, three fragments which give rise to the second transverse ciliated band of the pupa. IIIa, IIIb, two fragments which give rise to the third transverse ciliated band of the pupa. OR, four fragments which give rise to the oral ring of the pupa. *ap*, apical thickening of the larva; *stom*, stomodaeum, which becomes the atrium; *mp*, madreporic pore.

The second ciliated ring arises from the union of three fragments, two being the remnants of the antero-dorsal processes, and the third is the remnant of the right prae-oral process derived from the right side of the prae-oral loop. The first ciliated ring which immediately surrounds the opening of the atrium is derived from the left prae-oral process (Figs. 398, 399, 400).

By the shrinkage of the prae-oral portion of the larva the opening of the atrium and the band surrounding it come to be situated at the anterior pole of the larva, and so the transient asymmetry of the metamorphosing period is removed, and the larva again attains perfect symmetry. It is now in the form of a barrel surrounded by five ciliated hoops, with the atrial opening at the anterior and the anal opening at the posterior pole, and is known as the **barrel-shaped larva,** or as the **pupa.**

Whilst these external changes have been going on, the hydrocoele ring has become completed by the union of the two ends of the hoop which existed in the larva. During the process of the shrinkage of the prae-oral part of the larva the hydrocoele hoop has rotated till its plane, instead of being parallel to the length of the alimentary canal, becomes situated at right angles to it. Then what was the posterior and is now the ventral end of the hoop grows under the oesophagus, whilst the other end grows over the oesophagus to the right, bends down and meets the ventral end beneath the oesophagus, and so the ring is closed. Before actual closure occurs the ventral end can be seen to grow out into an inwardly directed lobe. This is the rudiment of the **Polian vesicle** (*Pol*, Fig. 401).

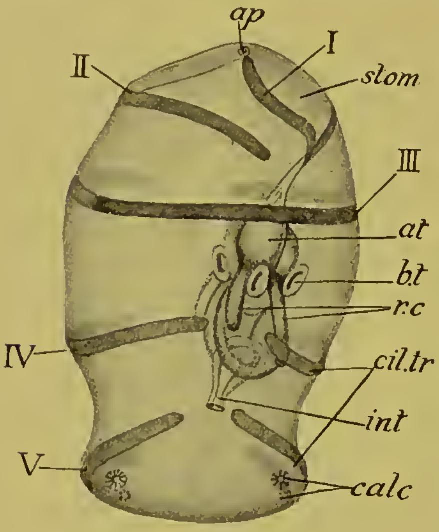

Fig. 400. — Metamorphosing larva of *Synapta digitata* viewed from the ventral aspect. (After Bury.)

Letters as in Figs. 398 and 399. In addition, *at*, atrium: a portion of the larval stomodaeum; *bt*, buccal tentacles; *cil.tr*, transverse hoops of cilia; *r.c*, vestigial radial canals.

The stone-canal opens into the ring in the interspace between the first and second of the smaller lobes, that is, between rudiments of the first and second radial canals, counting as first lobe the one most anteriorly situated, in the stage where the plane of the hoop is parallel to the long axis of the larva. Therefore the stone-canal opens into the ring in the same position as in the Asteroidea and Ophiuroidea. With the closure of the ring the peri-oral coelom also forms a complete circle, and the ventral horn of the left posterior coelom extends completely over to the right and round to the dorsal surface, where it grows towards the main portion of the left posterior coelom, which we may term its dorsal horn. The two horns are separated only by the anterior coelom and its extensions, the stone-canal and the pore-canal, just as is the case in Asteroidea, Ophiuroidea, and Echinoidea.

At the same time the oblique mesentery, separating left posterior coelom and right posterior coelom, becomes largely broken down, and this is also what occurs in Asteroidea. The longitudinal mesentery of the adult is not, as Semon assumed, identical with the space separating right and left posterior coelomic sacs in the larva, but only partly corresponds to this; it partly represents the space, filled with gelatinous connective tissue, which separates right and left horns of the left posterior coelom. The ciliated ring, which is invaginated, seems to give rise to the nerve-ring and to the ectodermal covering of the first five atrial tentacles, which, as they increase in length, protrude into the cavity.

According to Lyman Clark (1898), in *Synapta vivipara* the mouth comes to protrude from the floor of the atrium as a papilla. This papilla grows upwards, and its margin fuses with the sides of the atrial cavity high up; and in this way a portion of the atrial cavity becomes completely enclosed in the form of a ring surrounding the mouth. This ring is the **epineural canal**, which overlies the ring nerve. There is no doubt that a similar process takes place in *Synapta digitata*. At the same time the rudiments of the **radial canals** (*r.c*, Fig. 400) grow out and bend backwards, and extend along the radii. In *Synapta vivipara*, according to Clark, the rudiments of the radial canals remain entirely vestigial, and never grow back along the radii.

The pupa swims about for a considerable time, and is only very gradually transformed into the adult. As the tentacles grow in length the atrial opening is widened, and they protrude freely to the exterior and develop their lateral branches. At this stage the pupa, as figured by Metschnikoff (1869), seems to have only four transverse hoops of cilia, so that one of the five must have disappeared. The intestine, which until now has projected straight backwards, develops a knee-like bend where it issues from the stomach, and the loop formed by this hinge grows forward till it nearly reaches the level of the hydrocoele. In this way the ascending and descending limbs of the intestine are formed.

The peculiar **otocysts** (*ot*, Fig. 401) which distinguish *Synapta* are developed as evaginations of the epineural canal. The **ring of calcareous ossicles** surrounding the oesophagus is developed from mesenchyme cells which come to lie between the oesophagus and the nerve-ring. By these cells first five radial pieces, and much later five interradial pieces, are formed. An accumulation of mesenchyme cells also clusters round the anterior coelom, and here forms the calcifications which are known as the **secondary** or **internal madreporite**. Thus the remnant of the anterior body-cavity is enclosed, and it becomes pierced by pores which communicate with the coelom and which are lined with ciliated epithelium. The primary pore-canal and the pore disappear.

Soon after the atrium has opened out and the tentacles have become protruded, the transverse ciliated bands disappear, and the pupa sinks to the bottom and takes up the life of the adult. The stage with five tentacles is called by Semon the **Pentacula** stage.

The development of the genital organs has been observed by Clark in *Synapta vivipara*. In this species the eggs, when ripe, burst the covering of the ovarian tube and fall into the body-cavity of the mother, and here they are fertilized. They develop very rapidly; the segmentation of the egg and formation of the layers is exactly like what it is in *Synapta digitata.* The embryo, however, never develops either the folded ciliated band of the Auricularia or the transverse bands of the pupa; it is an oval organism uniformly ciliated all over. The stomodaeum arises on the ventral side, and becomes displaced to the left and then to the anterior pole. The larval anus disappears early, and the anus is re-formed later in development. The **genital organ** arises as a thickening of the epithelium covering the right hand side of the mesentery carrying the stone-canal, *i.e. from where the right horn of the left posterior coelom impinges on the anterior coelom*; the enlarged cells burrow into the gelatinous substances of the mesentery, there form a spherical mass which grows out into the genital tubes, and later forms its own duct leading to the exterior.

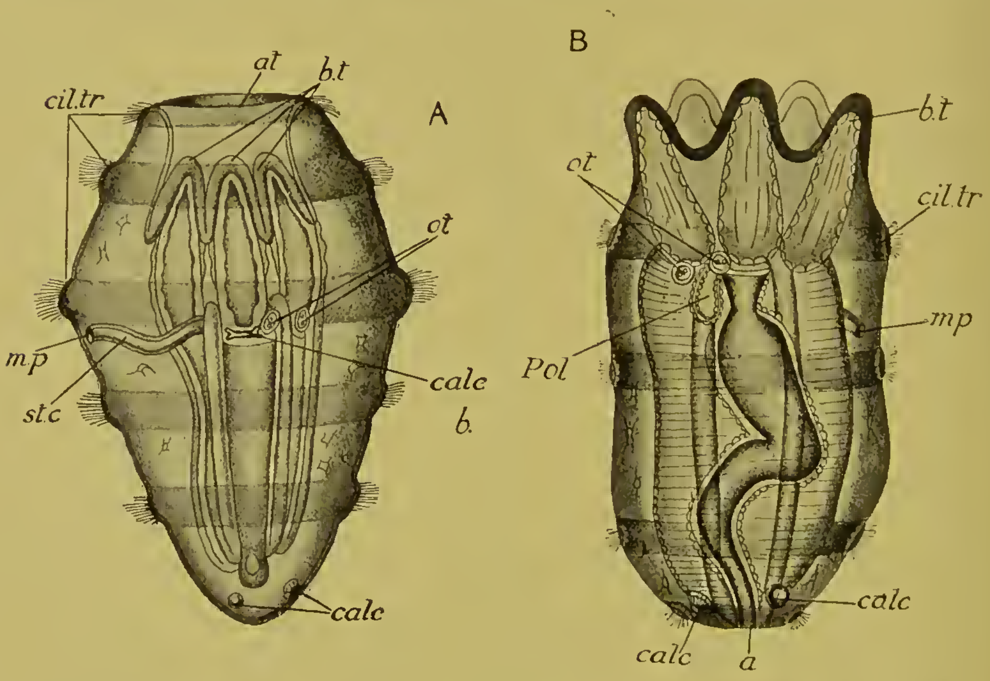

FIG. 401.—Pupa of *Synapta digitata* in two stages of development. (After Metschnikoff.)

A, before the buccal tentacles have been protruded from the atrium; note the five transverse ciliated bands. B, after the buccal tentacles have been protruded from the atrium; note the four transverse ciliated bands. Letters as in previous figure and in Fig. 398. In addition, *at*, atrium, *i.e.* larval stomodaeum; *calc.b*, rudiment of buccal skeleton; *ot*, rudiment of otocyst; *Pol*, rudiment of Polian vesicle; *st.c*, stone-canal.

Beyond all doubt the process of the development of the genital organs is the same in *Synapta digitata.* Stages in the development of the genital organs of *Cucumaria glacialis* have been observed by Mortensen, who confirms Clark's account (1904). The spot where the genital cells make their appearance is therefore the same in

Holothuroids as in Asteroids, Ophiuroids, and Echinoids. The Pentacula stage is soon passed, since accessory tentacles arise just over the spots where the radial canals are formed in *Synapta digitata*, and, in *S. vivipara*, from the rudiments of these canals. Thus we have a ten-tentacled stage, and the full adult condition is attained by the development of accessory tentacles in the dorsal interradii.

OTHER HOLOTHUROIDS

The only other of the species whose development exhibits an Auricularia larva, about whose life-history anything is known, is *Holothuria tubulosa*. For this we are indebted to Selenka (1876), who secured great numbers of adult individuals, and had the luck to obtain a ripe male and female which spawned spontaneously, and in this way a natural fertilization was obtained. The egg segments with great regularity, and gives rise to a spherical blastula of about 200 cells, which acquire cilia; the embryo then escapes from the egg-membrane and becomes a larva in the blastula stage, as in Asteroidea, Ophiuroidea, and Echinoidea—not, as in *Synapta*, in the gastrula stage. Then one side of the blastula becomes thicker than the rest, and becomes flattened. On this flattened surface the archenteric invagination appears, and from its apex, even when slightly developed, copious mesenchyme cells are given off.

The archenteron only projects a short distance into the blastocoele, so that there is a large prae-oral section of the larva. From the apex of the archenteron a large coelomic vesicle is given off, and the longitudinal ciliated band becomes established in the usual way, and then, afterwards, a vertical outgrowth is developed which forms the pore-canal; so that in this respect also *Holothuria* agrees with other Echinoderms and differs from *Synapta*, in which the pore-canal and madreporic pore are formed before the coelom has separated from the archenteron. The stomodaeum appears on the ventral surface as usual, and so the gut is completed, but the intestine bends forward as it does in other Echinoderm larvae. The coelomic vesicle divides into anterior and posterior portions, and the latter divides into right and left halves, just as in *Synapta*. The hydrocoele develops five lobes, but beyond this stage Selenka could not rear the larvae—a fact much to be regretted, as the development, so far as it goes, shows signs of conforming in every way more closely to the type exhibited by other Echinoderm larvae than does that of *Synapta*.

The development of *Cucumaria planci* has also been worked out, so far as external features are concerned, by Selenka (1876), and his account, in which there are many errors, has been corrected by Ludwig (1891). The eggs were obtained by spontaneous spawning, and a natural fertilization resulted. The segmentation is regular and results in the formation of a regular spherical blastula, although the eggs contain much yolk. Development is complete in about twelve days, but an Auricularia larval stage is never developed, and the larva in its early stages takes no food. The mesenchyme appears to be formed in the blastula stage, as in Echinoidea and Ophiuroidea, from one side of the blastula wall; it is this area which is later invaginated to form the archenteron. The coelom is separated off and divides exactly as it does in *Holothuria* and *Synapta*. Then the stomodaeum is formed, and the cilia are reduced so as to form five transverse rings. Were it not that a prae-oral region is retained which is covered with cilia, also an anal field in the neighbourhood of the anus, we might compare the larva to the pupa of *Synapta*.

Ludwig was able to determine that the covering of the buccal tentacles, and also the nerve-ring which connected their bases, took their origin from the ectoderm lining the larval stomodaeum. The tentacles arise from the radial canals and protrude into an atrium which is the larval stomodaeum. These radial canals are the first and only lobes developed from the hydrocoele ring. As the prae-oral lobe diminishes the mouth is gradually shifted to the anterior pole, and the cilia disappear. The paired tube feet appear on the radial canals, first on the mid-ventral one, and then, much later, on the lateral canals, and lastly on the dorsal one (Fig. 402). Unpaired tube feet seem not to be developed, *i.e.* the tips of the radial canals do not protrude as azygous tentacles. The ectoderm covering the tube feet is developed as nervous discs before the outgrowths from the radial canals appear. As soon as the first tube feet have developed the young *Cucumaria* sinks to the bottom and begins its creeping life.

An examination of young imago-stages of *Cucumaria*, just after the metamorphosis is complete, yields many most interesting results. Ludwig was able to keep the young *Cucumaria planci* alive for four months, and we ourselves have examined a series of young *Cucumaria* from the Antarctic (1912). The pore-canal is lost, but there is an anterior coelom embedded in the body-wall in which the stone-canal ends. This anterior coelom was seen by Ludwig, who, however, regarded it as a secondary evagination of the stone-canal. The walls of the atrium, *i.e.* the larval stomodaeum, have split into five valves (*v*, Fig. 402), and each valve is supported by a calcareous plate. A perfect cuirass of overlapping calcareous plates is found everywhere in the skin. The alimentary canal is still straight, but as the animal grows older the intestine lengthens and becomes thrown into a forwardly directed loop, and ciliated pores appear connecting the anterior coelom with the body-cavity, and in this way the secondary or internal madreporite is formed.

On reviewing the comparatively disconnected series of facts which we have just considered, it is clear that the larva of Holothuroidea, in spite of its external similarity to the Bipinnaria, is profoundly dissimilar when its internal anatomy comes to be considered. Thus, though both types of larvae possess a prae-oral lobe, that of Bipinnaria contains extensions of the left and right coelomic sacs, whilst that of Auricularia is filled with mesenchyme. The mode of segmentation of the coelom is widely different in Auricularia from what it is in Bipinnaria. On the other hand, the retention of the larval mouth and its shift towards the left, as seen in Holothuroidea, is not found in Asteroidea or Echinoidea, and is paralleled only by the development of the Ophiuroidea.

The growth of the buccal tentacles into the atrium, *i.e.* the larval stomodaeum, recalls what occurs in Ophiuroidea and in Echinoidea, if our conception of the amniotic space of the latter as a separated portion of the stomodaeum be admitted; but there is an important difference, *for the primary tentacles of Asteroidea, Ophiuroidea, and Echinoidea are the tips of the radial canals, but the tips of the radial canals of Holothuroidea do not protrude into the atrium, only their basal branches, the buccal tentacles, do so.* More light on this interesting difference will be obtained when the complete history of *Cucumaria saxicola* is worked out. This species, which is very closely allied to *Cucumaria planci*, has been reared through the metamorphosis by the authorities of the Plymouth Biological Station.

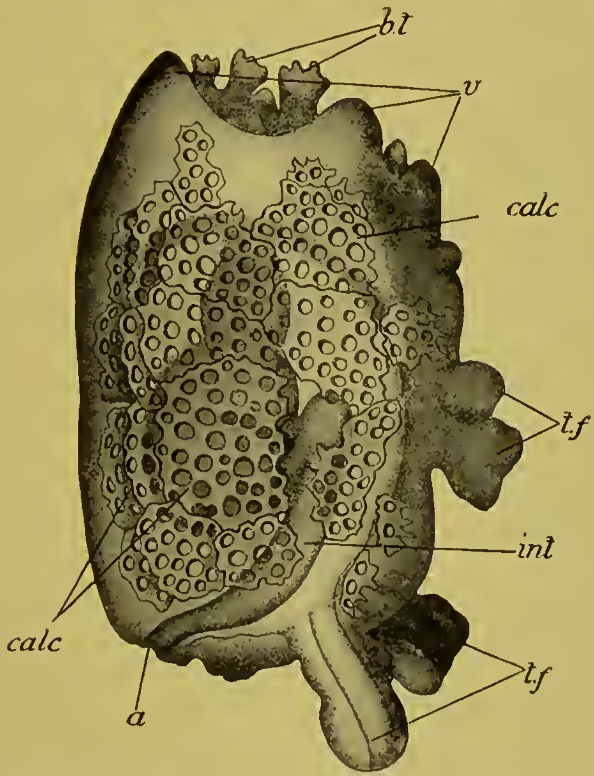

Fig. 402.—Post-larval stage in the development of *Cucumaria saxicola* viewed from the side. (Original.)

b.t, buccal tentacles; *t.f*, paired tube feet; *v*, valves into which the atrium splits.

The adult anatomy of a Holothuroid suggests the view that the group may have been derived from early Echinoidea in which the test consisted of plates movable on one another. This view is strengthened by the complete armour of plates in which the young *Cucumaria* is enveloped, and which gradually become separated from one another as the animal grows older, owing to the more rapid growth of the interstitial tissue; the manner in which the intestine grows after metamorphosis is complete also resembles what occurs in the Echinoid.

But if Echinoidea and Holothuroidea have diverged from a common stock, then the Echinoid larva has been much modified since that time in its external appearance; whilst in the Holothuroid larva the internal asymmetry in the development of the coelom has been pushed back to a very early period of development.

CRINOIDEA

The group of the Crinoidea is perhaps the most interesting division of Echinodermata because it is the dying remnant of the class Pelmatozoa, a class which once was represented by an enormous number of forms, and by such crowds of individuals that their skeletons form mountains of limestone in certain localities.

The development of only a single species, viz. *Antedon rosacea*, is known, and this species has yolky eggs, which only develop into a free swimming larva on the fifth day. This larva has a very brief free life, during which the mouth does not communicate with the stomach, and, consequently, no food is taken in. We cannot, therefore, compare this larva directly with such larvae as the Bipinnaria, Ophiopluteus, Echinopluteus, and Auricularia, but rather with the larva which emerges from such yolky eggs as those of *Solaster* and *Cribrella*.

Although *Antedon rosacea* is confined to the Western Atlantic and to the Mediterranean, other species of *Antedon* occur all over the world, and there is no reason to think that the development of any of these differs in any important respect from that of *Antedon rosacea*.

Leaving aside the earlier authors, such as Carpenter (1866), who described the external features of the development, we find that we have only to take account of the work of Bury (1888) and of Seeliger (1892), who investigated the development by means of modern methods. These two workers used practically the same methods, and their results happily confirm one another in all but unimportant details. Perrier (1880) deals, strictly speaking, with the post-larval development. As his work is earlier than that of Bury and Seeliger, and as, moreover, his methods are not so perfect and his starting point is later, it is quite obvious that he has not been so successful in unravelling the complex relations of the organs in the just metamorphosed larva, as they have been. To Bury belongs the credit of being the first to determine with accuracy the real course of Crinoid development. Seeliger, who came after him, did little but confirm his results. Both Bury and Seeliger obtained their material by the natural fertilization of the eggs.

Antedon, unlike Holothuroidea, lives in the open and can usually be obtained, when it is found at all, in enormous numbers by means of the dredge. At proper seasons of the year, ranging from May in the Mediterranean to July in the Clyde, a few ripe males and females are certain to be included in the haul. These, when placed in clean sea-water in the aquarium, will emit sperm and eggs early in the morning, about 7 A.M.

Both Bury and Seeliger found the best preservative to be a concentrated solution of corrosive sublimate in sea-water, to which a small proportion (Bury used $\frac{1}{3}$, Seeliger $\frac{1}{10}$) of glacial acetic acid has been added. For the study of the development of the calcareous plates the embryos must be preserved in spirit alone.

The fertilized eggs adhere to the under side of the pinnules of the parent by means of a glutinous egg-membrane, and from these eggs free-swimming larvae emerge on the fifth day.

The fertilized egg divides into two and then into four equal segments; in the 8-cell stage, however, the four blastomeres at the animal are smaller than the four at the vegetative pole. The **blastocoele** appears as a narrow central separation of the blastomeres in the 4-cell stage, which in the 8- and 16-cell stages is wider at the vegetative end. In the 32-cell stage it becomes closed at the animal end by the displacement of the blastomeres; in this stage the vegetative pole is surrounded by eight specially large blastomeres. In the 48-cell stage these eight blastomeres approach one another and close the opening at the vegetative pole. These eight blastomeres, however, subsequently divide, so that when 128 cells are formed and the complete blastula stage is attained no difference in the size of its component cells can be detected.

The **blastula** stage is attained in about eight hours. Then it becomes converted into a **gastrula** by the appearance of a pit on its surface and the invagination of a portion of its wall to form the lining of the **archenteron**. This pit or **blastopore** is not circular in outline but slit-like, and the long axis of this slit is at right angles to the plane of symmetry of the gastrula, which is no longer quite spherical but slightly elongated in one direction. The slit becomes crescentic, with the horns curved backward, but the archenteron is curved forward.

As soon as the archenteron is partly formed numerous **mesenchyme** cells are given off from its free apex and wander into the blastocoele. These cells arise in two ways; either a cell in the wall of the archenteron buds off a mesenchyme cell, or else it escapes bodily from between its neighbours in the archenteric wall. As the invagination proceeds the blastopore becomes reduced in size till it forms a small pore, and finally, when development has gone on for thirty-six hours, it is closed, although a groove marks for some time the spot where it existed.

The archenteron is flattened in the plane of the blastopore, *i.e.* in the transverse plane. In the second night, *i.e.* in about forty hours, it becomes constricted by a ring-like groove into an anterior and a posterior vesicle (Fig. 403, B). The latter becomes very much extended transversely, and narrowed in the middle, where it is embraced by two horns of the anterior vesicle, a dorsal and a ventral one, which grow backwards and surround the middle portion of the posterior vesicle like a ring.

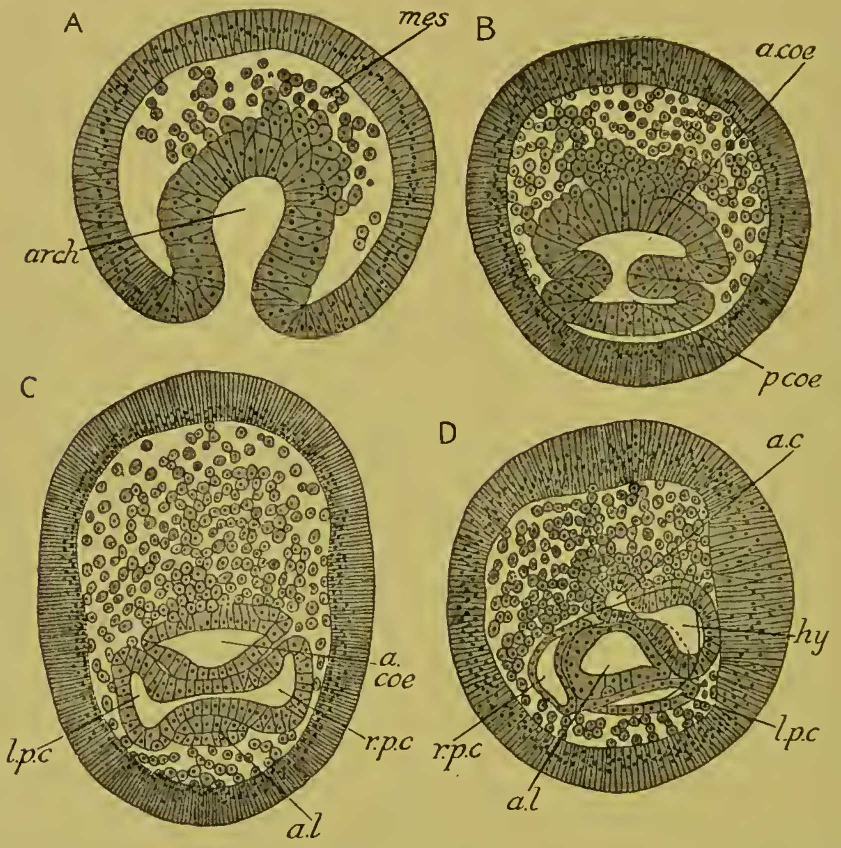

Fig. 403.—Early stages in the embryonic development of *Antedon rosacea*. (After Seeliger.)

A, sagittal section through an embryo twenty-six hours old in the gastrula stage. B, longitudinal frontal section through an embryo forty-eight hours old, to show the division of the archenteron into two coelomic vesicles. C, longitudinal frontal section through an embryo fifty-seven hours old, to show the belated formation of the alimentary canal. D, longitudinal sagittal section through an embryo seventy-five hours old, to show the complete division of the coelom and the formation of the hydrocoele. *a.c*, anterior coelom; *a.coe*, anterior primary division of the coelom; *a.l*, rudiment of alimentary canal; *arch*, archenteron; *hy*, hydrocoele; *l.p.c*, left posterior coelom; *mes*, mesenchyme; *p.coe*, posterior primary division of the coelom; *r.p.c*, right posterior coelom.

During the third day the embryo, which is now oval since it has grown more quickly along one diameter than the other, acquires a series of transverse ciliated rings which at first sight remind us of those of the Holothurian pupa, for there are five of them formed, and in addition an apical tuft of long cilia termed the apical field. The first of the five rings surrounds this field but is incomplete ventrally; the rest, however, are complete. The ectoderm cells constituting these rings are in active division. Some of the cells constituting the apical field lose their cilia and retreat to the bases of their neighbours, and here form ganglion cells; in this way an **apical plate** comparable to the apical plate of the Trochophore larva is formed, from whence a cord of nerve fibres extends down on each side of the ventral surface of the larva.

Whilst these changes are going on the anterior vesicle of the archenteron becomes constricted into a narrow, elongated dorsal portion and a spherical ventral portion. The former is the rudiment of the **anterior coelom,** the latter of the **hydrocoele.**

During the fourth day the dorsal and ventral horns, which grew back from the anterior vesicle, unite and form the **gut,** which thus at first has the form of a ring surrounding the narrow middle part of the dumb-bell-shaped posterior vesicle. The narrow part of the dumb-bell becomes a solid cord of cells and then disappears, and the ring-shaped gut becomes a sac by the filling up of the cavity of the ring. The right and left halves of the posterior vesicle become the **right and left posterior coeloms** respectively. These shift slightly one from another so that the right extends slightly on to the dorsal side of the embryo, the left on the ventral surface.

The anterior coelom, just after it separates from the hydrocoele, sends out on the right side a small outgrowth. This is probably the rudiment of a right hydrocoele. The anterior coelom becomes completely constricted off from the hydrocoele, and both are divided from the definitive gut. The **primary madreporic pore** is formed by an outgrowth from the hinder end of the anterior coelom meeting the ectoderm on the left near the ventral line.

The larval **stomodaeum** then appears as a thick-walled invagination on the ventral surface of the larva between the second and third ciliated rings, which it presses apart. It is therefore evident that these ciliated rings are not strictly comparable with either those of the *Cucumaria* larva or the pupa of *Synapta.* The two cords of fibres proceeding from the apical nervous system pass down its sides. Behind the apical field and in the gap of the first ciliated band is a small glandular pit in the ectoderm. This is the **fixation pit,** by which the larva eventually attaches itself to the substratum. The floor of the stomodaeum does not come into contact with the wall of the gut, so that the larval alimentary canal, which is devoid of an anus since the blastopore became closed, does not acquire a mouth, but it does come into close contact with the hydrocoele which is flattened against it.

During the fifth day the embryo escapes from the egg-membrane as an oval larva and swims about by means of its ciliated bands. This phase of its development may last from a few hours to four or five days. About this time the calcareous ossicles can be made out, embedded in the mesenchyme. Of these we have two oblique rings of five ossicles encircling the hinder part of the body—the hinder ring is known as the **oral ring of plates,** the front one as the **basal**

ring of plates. In front of the latter are a group of three very small **under-basal plates**, whilst in front of these there is a linear series of **eleven columnals**, each a crescent-shaped ossicle with the concavity directed ventrally.

At the conclusion of free-swimming life the larva fixes itself by its adhesive pit to the substratum, and its anterior portion, surrounded by the columnals, becomes converted into the **stalk** of the young Crinoid; but if the free-swimming life lasts longer than one day considerable readjustment of the internal organs takes place before fixation occurs.

The shift of the right and left posterior coeloms on each other increases till the right has become almost completely dorsal and the left ventral, but the latter does not extend ventrally between hydrocoele and ectoderm. In addition to this dorso-ventral shift, a shift in an antero-posterior direction takes place, so that the left coelom shifts posteriorly until it covers the posterior end of the gut, whilst the right coelom shifts anteriorly. If we disregard this second shift and consider the relative positions of the right and left posterior coeloms, when they have just separated from one another, as marking the right and left sides of the embryo, then we should say that the hydrocoele, instead of being mid-ventral, is well up on the left side, and so is the primary madreporic pore; the larval stomodaeum will also then be an organ of the left side. When the second, the antero-posterior shift takes place, if the description given above has been followed, it

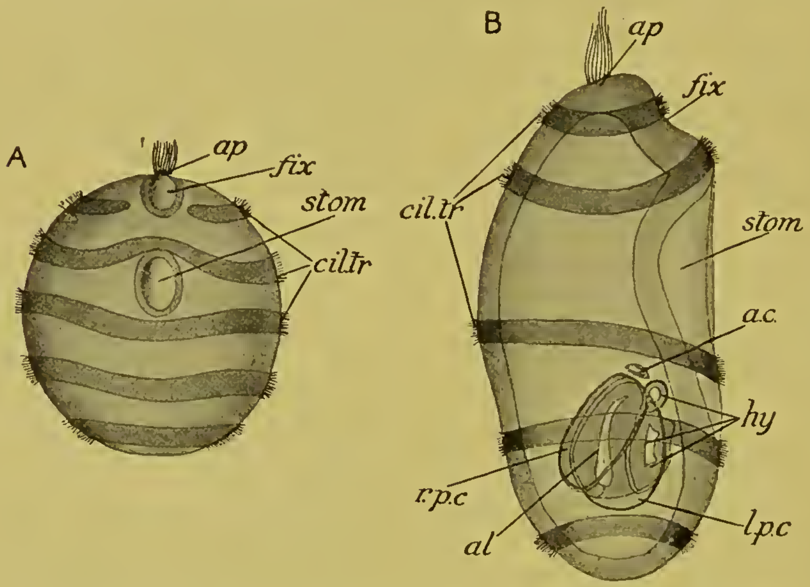

FIG. 404.—External views of the embryo and larva of *Antedon rosacea*, showing the mutual relations of stomodaeum and ciliated bands. (After Seeliger.)

A, embryo just before hatching, ventral view. B, larva one day after hatching. Letters as in previous figure. In addition, *ap*, apical plate; *cil.tr*, transverse bands of cilia; *fix*, fixing disc; *stom*, larval stomodaeum—the rudiment of the future vestibule.

will be seen that the **longitudinal mesentery** separating the left and right posterior coeloms runs in a curve, the concavity of which is directed forwards.

During free-swimming life the hydrocoele, which lies in a concavity of the gut, takes on a hoop-like form with the concavity directed towards the left; later it rotates so that the concavity is directed forwards, and it is then seen that the right limb is thicker than the left. The five primary lobes, the rudiments of the future **radial canals**, arise as ventrally directed outgrowths. The stomodaeum closes behind so that its opening becomes narrowed. The right posterior coelom sends forward two dorsal diverticula which lie above the anterior coelom. These two fuse with one another ventrally, but their lateral walls, where they are in contact with one another, form the **longitudinal mesentery** of the right coelom. From their apices spring five narrow diverticula whose openings are arranged in a circle; these extend forwards to the apical field and constitute the rudiment of the **chambered organ** (Fig. 406, B). The left coelom extends so far

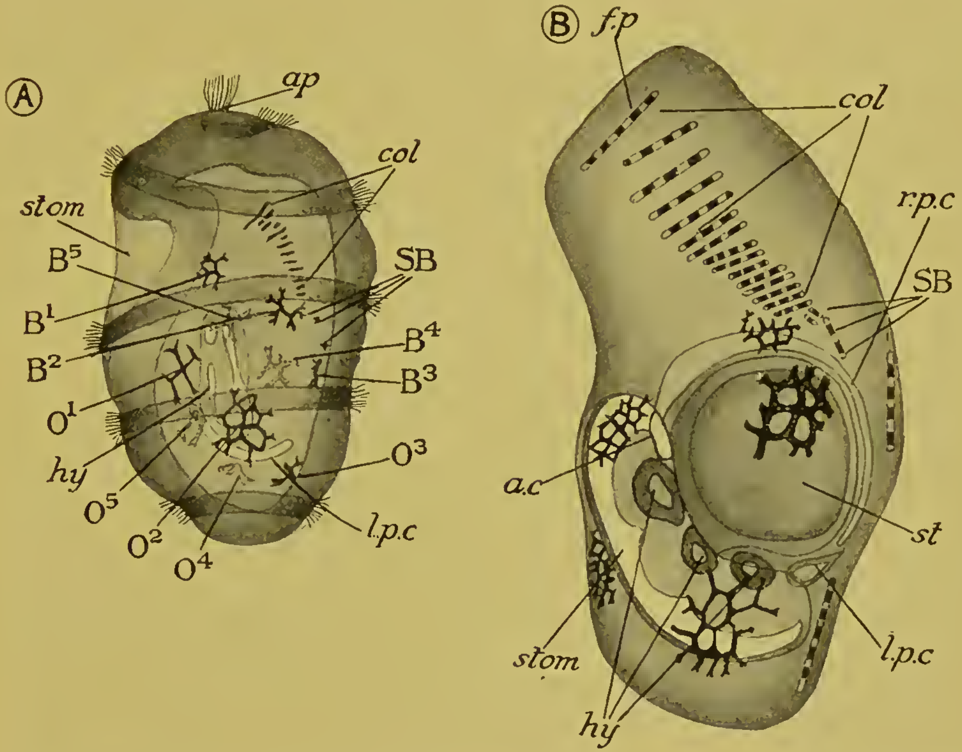

Fig. 405.—External views of larvae of *Antedon rosacea*—at the time of hatching and after fixation, in order to show the development of the calcareous ossicles. (After Seeliger.)

A, larva at the time of hatching. B, larva two days after hatching—fixed. Letters as in previous figure. In addition, *col*, rudiments of the columnal plates; B^1-B^5, rudiments of the five basal plates; *f.p*, foot-plate; O^1-O^5, rudiments of the five oral plates; SB, rudiments of the under-basal plates.

over to the right posteriorly, that it becomes horse-shoe shaped with the concavity of the horse-shoe directed forwards.

If the free-swimming stage lasts long the rudiment of the **oesophagus** appears as a solid peg-like outgrowth of the stomach, which grows between the two limbs of the hydrocoele towards the floor of the stomodaeum; and the rudiment of the **stone-canal** also appears as an outgrowth from one end of the hoop-shaped hydrocoele. According to Seeliger the original connection between anterior coelom and hydrocoele was in the middle of the hoop, as in *Ophiura brevis*, and for that matter in *Asterina gibbosa* also. The mesenchyme in the anterior portion of the larva, towards the centre, consists of closely packed rounded cells, and in these the calcareous **columnals** are formed, but towards the periphery the mesenchyme forms elongated fibre-like cells, which later form muscles and fibres in the stalk of the young Crinoid. Each lobe of the hydrocoele develops a pair of lateral lobes, and the original five lobes begin to protrude into the stomodaeum as free **tentacles** (Fig. 405, B).

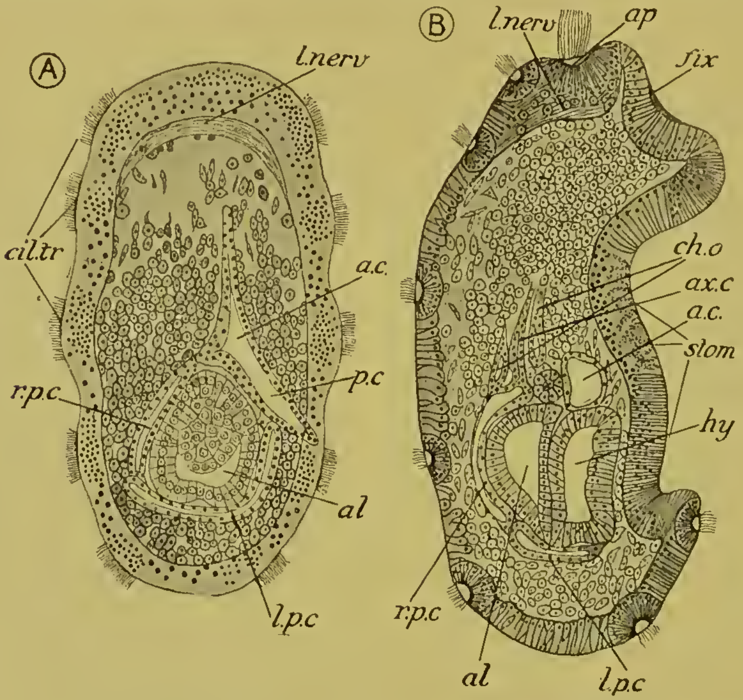

Fig. 406.—Longitudinal sections through free-swimming larvae of *Antedon rosacea*. (After Seeliger.)

A, section in the sagittal direction, but not in the sagittal plane, through a larva which has just been hatched. B, median sagittal section through a larva which has been hatched for twenty-eight hours and is about to fix itself. Letters as in two previous figures. In addition, *ax.c*, one of the axial prolongations of the right posterior coelom; *ch.o*, rudiments of the chambered organ—outgrowths of the right posterior coelom; *l.nerv*, fibres of the larval nervous system.

Then the larva, having found a suitable spot, fixes itself to the substratum by the secretion of the prae-oral pit. At first it lies with its length parallel to the substratum, but it soon becomes erected so as to stand at right angles to the substratum. The stomodaeum becomes completely closed from the exterior, and so resembles the amniotic space of the Echinopluteus larva; it rotates backwards along the ventral surface till it comes to occupy the posterior pole of the larva, the body of which becomes differentiated into a narrow anterior portion, the stalk, and a posterior broader part, the cup or **calyx**. This calyx becomes pentagonal in section, and so the radii of the Crinoid are marked out, and the lobes of the hydrocoele soon come to correspond with the sides of the pentagon.

The fixation pit flattens out to form a fixing disc, which becomes supported by a calcareous **foot-plate**. The cilia are shed, and the cells which formed the ciliated bands secrete a cuticle and then retreat from the surface to some extent, touching it only by thin prolongations; processes from the mesenchyme cells extend up between them and it soon becomes absolutely impossible to discriminate between ectoderm and mesenchyme. The same fate befalls the ectoderm cells forming the intermediate areas, indeed it sometimes happens to them before fixation. The ectoderm lining the stomodaeum, which has now become the closed **vestibule**, however, undergoes none of these changes. Where it covers the hydrocoele and the tentacles it is a thick syncytium, elsewhere it is a thin layer of flattened cells.

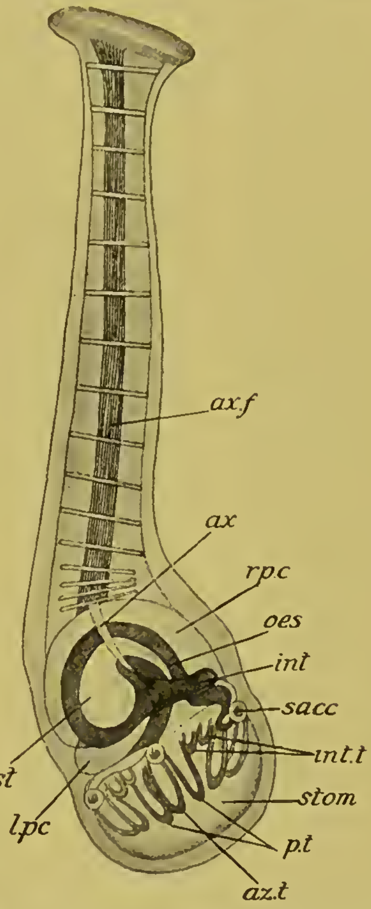

FIG. 407.—Fixed larva of *Antedon rosacea*, three and a half days after hatching, viewed from the side—decalcified. (After Seeliger.)

ax, axial organ containing the genital stolon; *ax.f*, axial band of fibres surrounding the chambered organ; *az.t*, azygous primary tentacle of the hydrocoele; *int*, rudiment of the intestine; *int.t*, inter-radial tentacles; *oes*, oesophagus; *p.t*, paired tentacles; *sacc*, rudiment of sacculus; *r.p.c*, right posterior coelom; *st*, stomach; *stom*, larval stomodaeum now become the vestibule.

The number of tentacles becomes raised to twenty-five by the appearance of another pair in each radius, situated below those already formed and apparently springing directly from the hydrocoele. The first pair of tentacles also come to spring directly from the hydrocoele ring, by the absorption into this ring of the base of the primary tentacle of which they were outgrowths. All these tentacles become long and protrude into the vestibule, but the last formed are shorter and not so extensile as the first formed. All the tentacles which belong to one interradius are connected by a common web of ectoderm.

The gut swells out into a globular form; wandering cells are budded from its wall, pass into its cavity and are absorbed. From its right side a small horizontally directed pouch grows out. This is the rudiment of the **intestine** which now grows from right to left, lying in the septum dividing the left from the right posterior coelom. The left posterior coelom has been carried back along with the stomodaeum or vestibule, and has now become thoroughly posterior or oral in position, whilst the right posterior coelom is now quite aboral. The main body of the left posterior coelom wedges itself in between stomach and hydrocoele, and it is henceforward known as the **oral coelom.** Between its two limbs lies the oesophagus. Its right limb also extends inwards beneath the gut so that the opening of the horse-shoe, instead of being directed forwards, is deflected to the left. The left horn of the right posterior coelom wedges itself in between the left posterior coelom and the gut, and here fuses with the right ventral horn of the left coelom, and the two cavities merge in one another. The right posterior coelom is now termed the **aboral coelom.**

On the right side the main body of the right coelom covers the left internally. The two partially coalesced anterior diverticula of the right coelom, by whose opposition the vertical mesentery is formed, extend away forwards and form a central canal surrounded by the five rudiments of the chambered organ. The walls of these rudiments have become thinner and their cavities swollen, especially at their bases, and so the **ampullae** of the chambered organ are formed. The anterior extension of the anterior coelom has disappeared, and this space becomes reduced to a small rounded sac on the ventral side, opening by the **primary madreporic pore** (*p.c*, Fig. 405, B).

The rudiment of the **genital organs** appears as the so-called **axial organ,** which is equivalent, although not exactly homologous, to the **genital stolon** of other Echinoderms. It appears as a line of enlarged cells on the left side of the longitudinal septum of the right coelom. In the groups so far studied it seems to arise as a proliferation of the wall of the left posterior coelom. This line or cord, becoming detached from the epithelium of the coelomic cavity, sinks into the gelatinous substance of the mesentery and then grows backwards, *i.e.* towards the calyx or cup; the hinder portion is in the condition of a thickening of the epithelium, whilst the front part is already sunk inwards.

The calcareous plates, *i.e.* the **orals** and **basals,** which in the free-swimming larva formed oblique circles round the body, now form transverse circles; the orals surrounding the vestibule, the basals the aboral coelom. Five **sacculi** (Fig. 407) are formed in the wall of the vestibule alternating with the orals. These sacculi are clumps of mesenchyme cells which become hollowed out and secrete a bright yellow pigment; they are probably of an excretory nature.

Next, the oral vestibule becomes opened to the exterior, the tentacles protruded, and the young Crinoid begins to feed. The

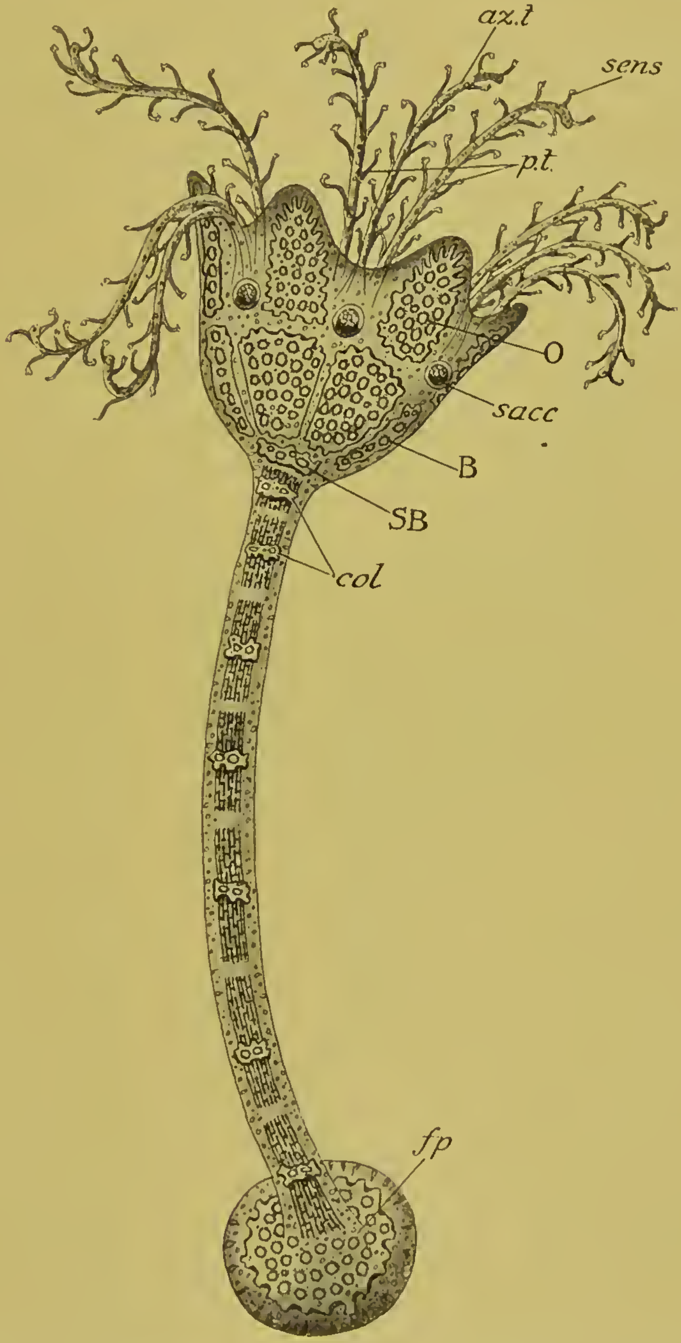

FIG. 408.—Fixed larva of *Antedon rosacea* in which the vestibule is open but in which no trace of the arms has yet appeared. (After Carpenter.)

Letters as in previous figures. *sens*, sense-organ on tentacle.

outer wall of the vestibule splits into five valves (*r.v*, Fig. 408), each of which is supported by an oral plate, and the tentacles are protruded between the valves. The epithelium lining the vestibule becomes thin, except just over the hydrocoele ring where it becomes thick and ciliated, and beneath this ring of thickened epithelium a **ring of nerve fibres** makes its appearance. The high ciliated epithelium and the nerve fibres are prolonged over the inner sides of the tentacles. Sense-organs are scattered over the surface of these tentacles. Each sense-organ consists of a conical prominence of elongated ectoderm cells. The tips of the cells forming the apex of the cone become elongated so as to form a tactile rod.

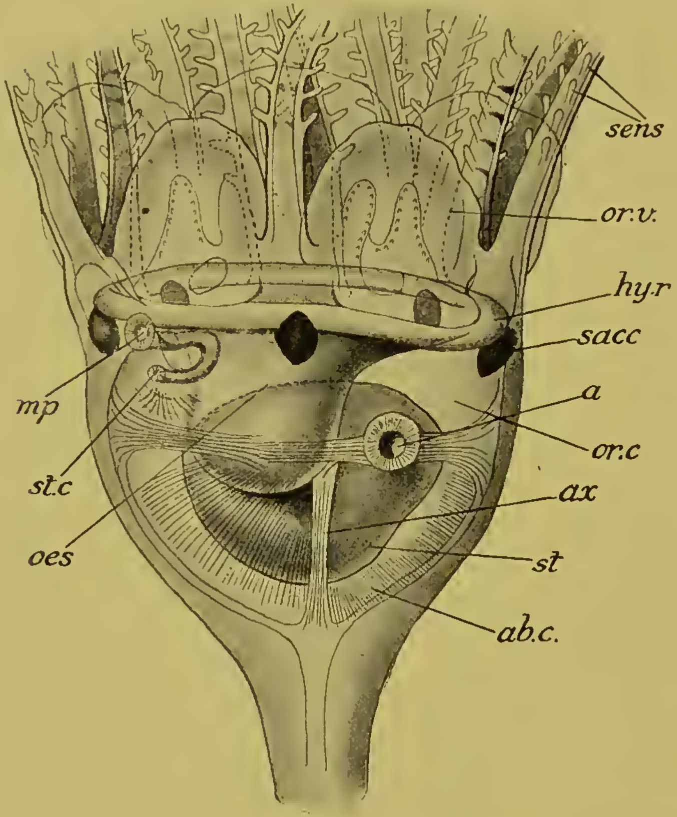

Fig. 409.—Calyx of larva of about the same age as that represented in Fig. 408, decalcified and cleared in order to show the internal structures. (After Ludwig.)

ax, axial organ containing the genital stolon; *ab.c*, aboral coelom; *hy.r*, hydrocoele ring; *m.p*, primary madreporic pore; *or.c*, oral coelom; *or.v*, oral valve; *st.c*, stone-canal.

The wall separating the aboral coelom and the anterior coelom thins out till it forms a flat membrane of epithelium, which is then absorbed and the two cavities which it separated become merged into one.

In this manner we reach the condition found in the adult Crinoid, where the stone-canal opens at one end into the general coelom, and the pore-canal takes its origin independently from this.

The "axial organ" becomes a tube, and the vertical mesentery of the aboral coelom breaks down into strands.

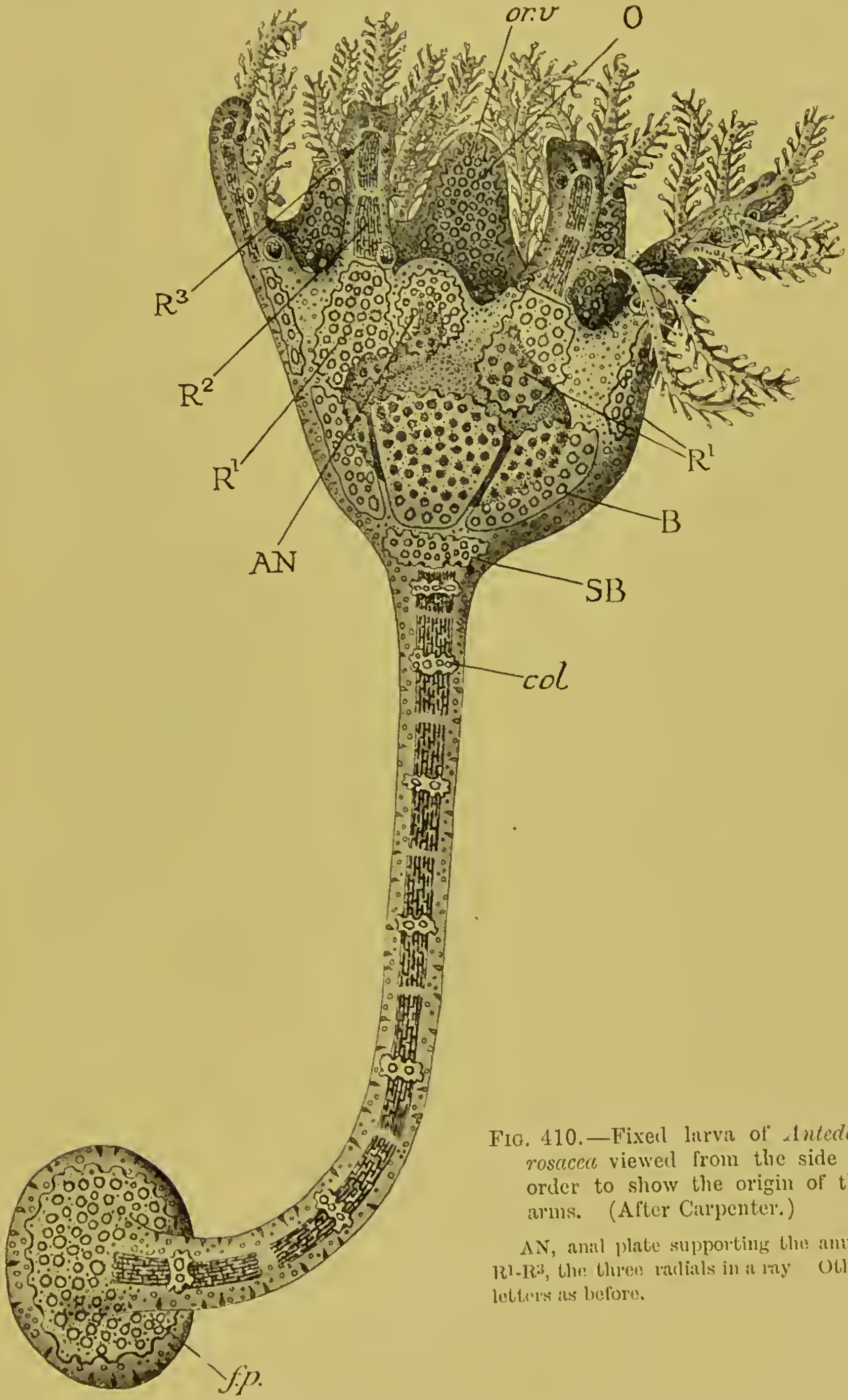

FIG. 410.—Fixed larva of *Antedon rosacea* viewed from the side in order to show the origin of the arms. (After Carpenter.)

AN, anal plate supporting the anus; R¹-R³, the three radials in a ray Other letters as before.

Beyond this stage Seeliger could not rear his larvae, and here Perrier's observations may be said to commence; some splendid figures are given by Carpenter (1866) of the stages described by this author. The first stage described by him is the fixed larva with the closed vestibule; following on this he has seen the growth of the **arms** of the young Crinoid. These arms arise as vertical upgrowths of the calyx alternating with the oral valves. They are supported each at its base by a new plate, the **primary radial** (R^1, Fig. 410), and as they grow in length **secondary** and **tertiary radials** are added (R^2, R^3, Fig. 410). To these incipient arms the median tentacle in each radius applies itself and becomes the **radial canal** of the arm (*r.c*, Fig. 411). When the arm forks the tip of the radial canal remains as an azygous tentacle at the fork, but two branches are

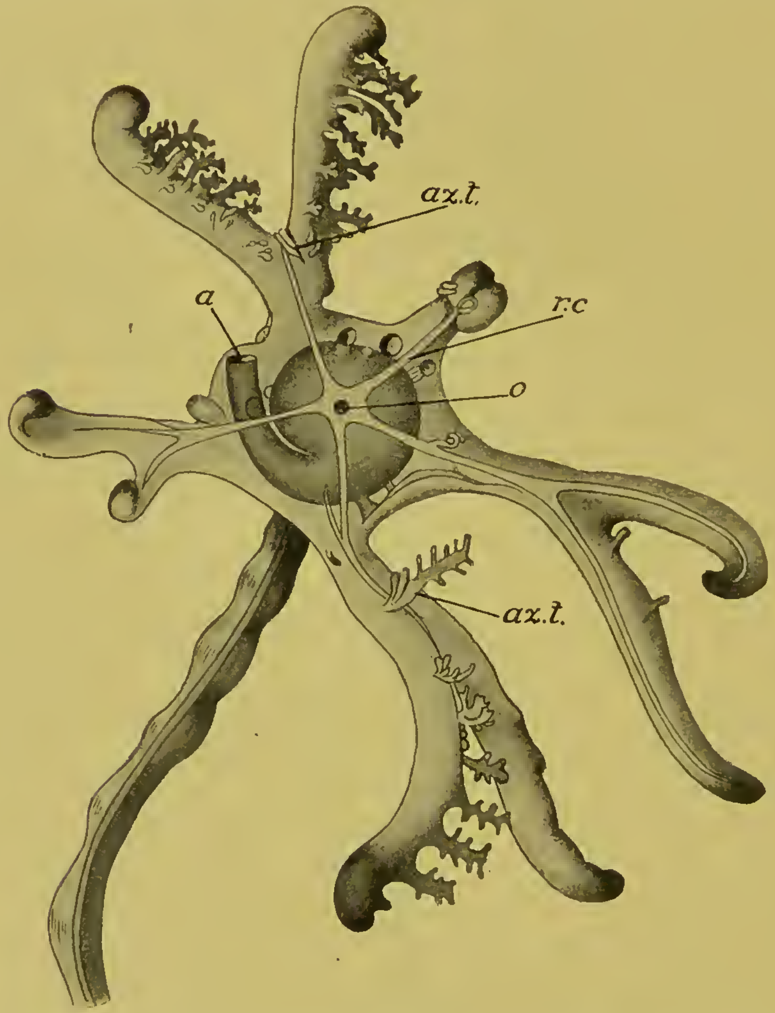

FIG. 411.—View of the calyx of a fixed larva of *Antedon rosacea* from the upper side, in order to show the adhesion of the lobe of the hydrocoele to the arm and the first dichotomy of the arm. (After Perrier.)

a, anus; *az.t*, azygous tentacle formed from the tip of the primary lobe of the hydrocoele; *o*, mouth; *r.c*, radial canal formed from the base of the primary lobe of the hydrocoele.

given off just below the tip which become the radial canals of the daughter arms. In the first dichotomy both arms are equal in size, but in each dichotomy subsequently one branch remains short and forms a **pinnule** but the other grows on and forks again, when the same process is repeated, so that the apparently single arm of the Crinoid is really a sympodium formed of a succession of the stronger members of successive dichotomies. As the arms grow they become more and more directed outwards, till from their original vertical they pass into a horizontal position.

Whilst these changes are proceeding **secondary stone-canals** are formed, at first one in each of the four interradii not occupied by the primary stone-canal. Each of them arises as two solid buds, one bud being formed in the outer wall of the coelom and the other on the hydrocoele ring. Each of these buds becomes hollowed out to form a vesicle. The external one forms a pore and a thin-walled sac communicating therewith, a replica of the anterior coelom in fact. The internal bud forms the new stone-canal which reaches and fuses with the vesicle. These new anterior coeloms soon fuse with the general coelom, as did the original one, and so the secondary stone-canals and the secondary madreporic pores both open into the general coelom and are disconnected from each other.

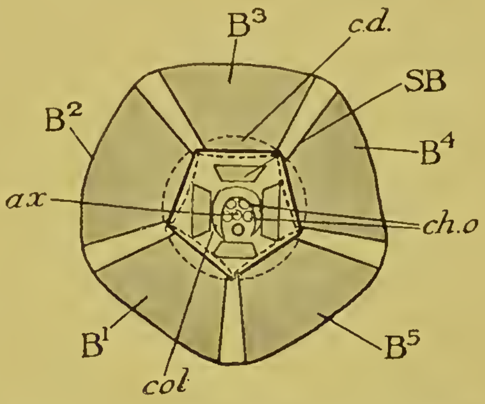

FIG. 412.—Map showing the mutual relations of the ossicles in the base of the calyx of a fixed larva of *Antedon rosacea* when the adult condition has been nearly attained. (After Seeliger.)

B^1-B^5, the five basal ossicles; SB, the sub-basals; *ax*, central canal containing the axial organ; *c.d* (dotted line), the outline of the adult centro-dorsal cell—the plates within this become fused together; *ch.o*, the five canals of the chambered organ; *col*, the uppermost columnal.

In later stages the new pores and stone-canals do not even reach one another, and eventually are formed irregularly and independently of one another, and so the adult condition is reached. The first branches of the stem, or **cirri**, are formed just when the first secondary stone-canals make their appearance. The canals of the chambered organ expand to form ampullae at the bases of these, repeating the primitive ampullae at the base of the stem; and a branch from the axial organ and the central canal grows out and traverses each ampulla, and forms a new central canal with its axial organ in the centre of each cirrus.

These ampullae are really the **brain-vesicles** of the all-important **aboral nervous system** which dominates the movements of the adult. The cells surrounding them, which are mesenchyme cells budded from their walls, become converted into the ganglion cells of this system. The nervous strands which proceed outwards from these centres are at first merely grooves or gutters in the internal walls of the calyx. They become shut off from the coelom and transformed into solid cords of cells which develop sheaths of nerve-fibres. The mesenchyme cells, by which they are surrounded, become calcigenous tissue, and thus the original plates of the young Crinoid are increased in thickness, and in this way the nerves come to lie in the centre of the radial and brachial plates of the adult. Exactly the same process takes place with regard to the cirri; each ampulla of the chambered organ gives off, as we have seen, a branch leading into the cirrus, and this branch develops a nervous investment.

The muscles connecting the stem-joints one with another, and the various ossicles of each cirrus are also formed from mesenchyme cells; and the same thing is true of the muscles connecting the arm-joints with one another. No embryological distinction can be perceived between the cells forming the dorsal elastic ligaments of the arm-joints, which straighten the arms, and the ventral flexor muscles which bend them, and we are confronted with the remarkable fact that nervous, muscular, connective, and skeletal tissues arise from cells of an identical origin.

Meanwhile the portion of the axial organ which is situated in the calyx has been growing. It becomes plexiform, its original single tube-like cavity being split into several cavities. Where its originally lower end reaches the wall of the stomach it gives off five branches, at first solid but soon becoming hollowed out, one of which grows into each arm and there forms the **genital rachis.**

According to Perrier, the septum dividing the oral and aboral sections of the coelom contained in the arm is a secondary formation, and has nothing to do with the original septum dividing oral and aboral coeloms from one another. This may be doubted, and a re-examination of this point would be desirable. The finished **genital organ** is nothing but the apex of the branch of the rachis which penetrates the pinnule. As in the case of other Echinoderms, the duct is produced by an outgrowth of the cells of the genital organ itself, which burrow their way to the outside.

By the secondary thickening of the radial plates, the primary ampullae of the chambered organ become completely overarched and shut off from the aboral coelom. The **under-basals** unite with the uppermost stem ossicle to form the **centro-dorsal ossicle,** on which numerous new cirri make their appearance. This centro-dorsal ossicle grows till it reaches the primary radials and shuts off the basals from the exterior. These unite to form a single piece, the **rosette plate,** which forms the roof of the chambered organ.

The young Crinoid in this stage, in which arms, pinnules, and cirri are developed, is termed a **Pentacrinoid** larva, from its resemblance to the genus *Pentacrinus*.

Soon after this the young Crinoid wrenches itself loose from the stalk, which parts just below the centro-dorsal, and so enters on its adult life, when it can swim freely from place to place, and can also attach itself by its cirri.

When we review the life-history which has just been described,

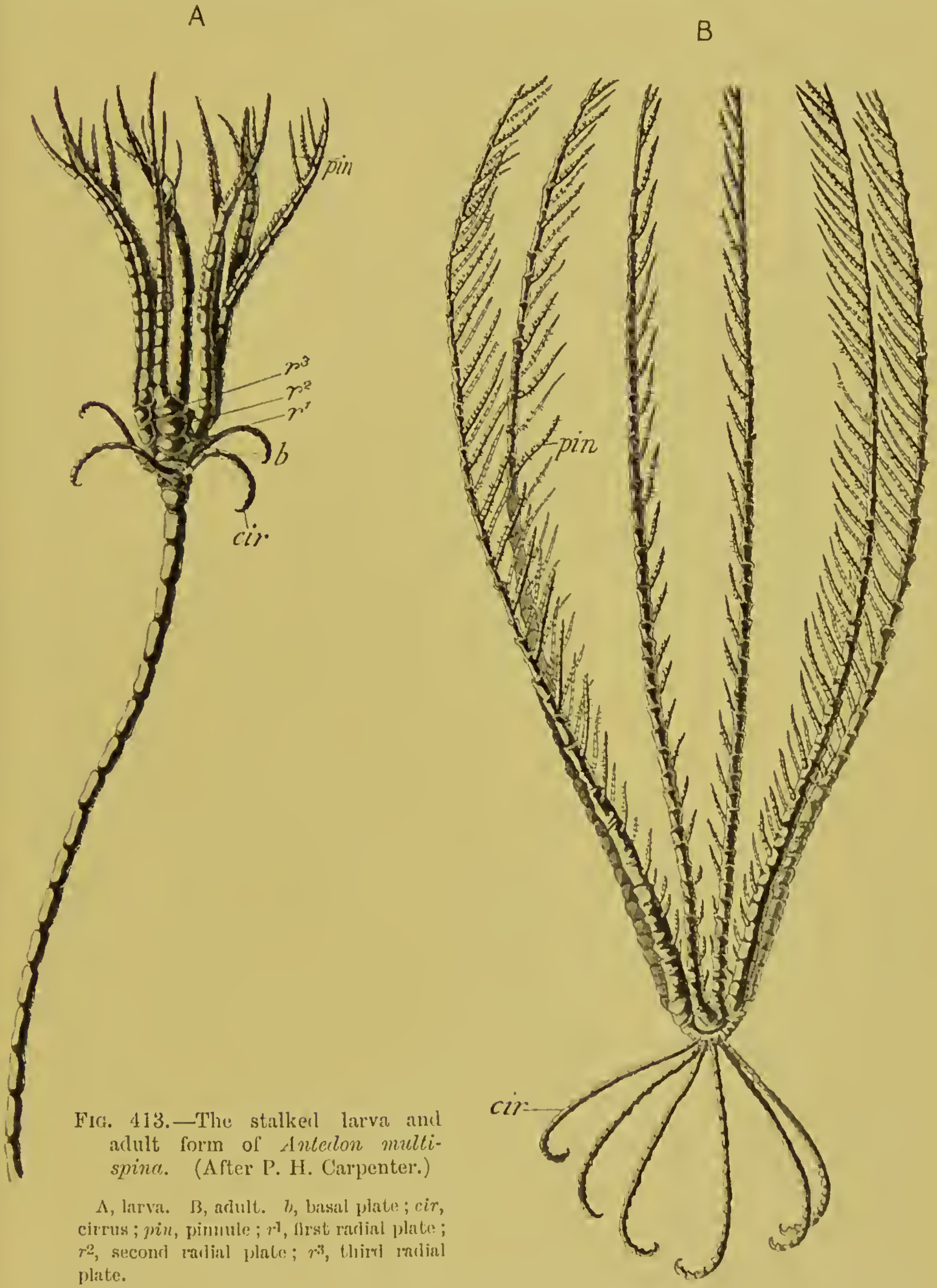

FIG. 413.—The stalked larva and adult form of *Antedon multispina*. (After P. H. Carpenter.)

A, larva. B, adult. *b*, basal plate; *cir*, cirrus; *pin*, pinnule; r^1, first radial plate; r^2, second radial plate; r^3, third radial plate.

we may note, first of all, that some of the stages through which the young Crinoid passes in its growth, till it loses its stalk, correspond to the permanent condition in other genera of Crinoidea, both living and extinct. Thus the stalk is retained throughout life in the living genera *Pentacrinus, Rhizocrinus, Bathycrinus,* and *Hyocrinus,* and in the vast majority of fossil forms. The basals remain exposed in *Thaumatocrinus, Rhizocrinus, Bathycrinus, Hyocrinus,* and in an enormous number of fossil forms. Lastly, in many fossil forms the under-basals are distinct and exposed, and the nerves running from the aboral nervous system, instead of being enclosed in the ossicles, run in open grooves in them.

ANCESTRAL SIGNIFICANCE OF LARVAE OF ECHINODERMATA

We may now pass in review the larvae of Echinodermata viewed as a whole. We may discount the characteristic features in the development of *Antedon,* which are obviously due to the yolky egg and incapacity of the free-swimming larva to take food, and regard it as tolerably certain that, if we were lucky enough to find a Crinoid with a small yolkless egg, that this would develop into a larva fundamentally similar to the larvae of Asteroidea, Echinoidea, Ophiuroidea, and Holothuroidea. These larvae agree in possessing as a locomotor organ a folded longitudinal ciliated band with prae-oral and anal loops; a V-shaped adoral ciliated band which apparently removes surplus food from the region of the mouth; an alimentary canal consisting of shovel-shaped buccal cavity (stomodaeum), oesophagus, globular stomach, and intestine; and a coelom, which originates as a pouch from the anterior end of the gut, which divides into right and left halves, and which communicates with the exterior by a ciliated canal opening on the dorsal surface to the left side of the middle line.

Now, when we find a group of larvae exhibiting so many characters in common, we conclude that they represent a common ancestor from which the groups to which they belong are descended. To this hypothetical ancestor we may give the name **Dipleurula.** Since the larvae in question, so far as their external features go, are perfectly bilaterally symmetrical, we may regard those of them whose internal organs exhibit the nearest approach to bilateral symmetry as, in this respect, retaining the characters of the ancestor. It follows that the larvae of Ophiuroidea are in this respect most primitive, for it is in every way likely that the internal asymmetry displayed so markedly by the Auricularia larva, and in much lesser degree by the Bipinnaria and the Echinopluteus, is an anticipation of the adult arrangement, where the organs of the left side overpower those of the right.

We may credit then the ancestral Dipleurula with a coelom which, on each side, was perfectly or imperfectly divided into three divisions: an anterior, a middle, and a posterior. The middle division was prolonged into lobed processes, covered externally with ciliated epithelium, which projected at each side of the mouth and produced currents which transported small pelagic organisms, on which the animal fed, to the mouth. In a word, the two hydrocoeles of the ancestor were in every way similar to the lophophoral arms of Brachiopoda, to the halves of the lophophore of Polyzoa and of *Phoronis*, and performed a similar function; and it must be remembered that all these lophophores consist of hollow protrusions of a special division of the coelom, clothed externally with ciliated epithelium. The peculiarity of the Echinoderm ancestor was the anterior coelom with its dorsal ciliated pore; this, when both hydrocoeles were equally developed, was also probably double.

No adult animal exactly like this supposititious ancestor at present swims in the seas, but there are many features of resemblance between the Dipleurula, whose many features have thus been reconstructed, and a simple Ctenophore, if we regard the **gastro-vascular canal system** as representing a coelom which is still in open communication with the digestive gut, represented in Ctenophores by the **infundibulum** or **funnel.** In Ctenophores the gastro-vascular canal system consists of an anterior section which is divided into a series of lobes underlying the ctenophoral canals, and which gives rise to the canal which leads to the base of the tentacle, and of a posterior section, the **paragastric canal,** which runs backwards to the posterior end of the animal; there is also an anterior unpaired canal given off from the upper end of the funnel which terminates in two pores at the anterior end of the animal. The primitive mouth is not yet divided into mouth and arms, so that the Ctenophore represents a more primitive condition than our hypothetical Dipleurula. Locomotion is effected by radiating bands of ciliated epithelium; and where they converge at the apical pole there is situated a median neuro-sensory patch of ectoderm, which is in every way comparable to the organ which appears in the Echinopluteus larva when it is three weeks old, and to the sense-organ at the apex of the Crinoid larva.

Now the arrangement of the ciliated ectoderm is very different in a Ctenophore from what it must have been in a Dipleurula; still, if we had before us the Dipleurula and the Ctenophore-like ancestor of Annelida and Mollusca, when the mouth and arms had become separated from one another, we should probably have regarded them as members of the same class of animals. We must remember the marked tendency of patches of ciliated epithelium to undergo rearrangement and reunion—as is shown by the production of transverse ciliated bands in the Auricularia, and in the Crinoid larva, and in the Echinopluteus of *Echinus esculentus,* and these transverse bands do not exactly correspond to one another in any of these three cases.

It is a fascinating and in every way a likely supposition, that there existed a class of simple marine animals, distributed all over the world and diversified into orders, genera, and species, and that the Ctenophore-like ancestor of Annelida and Mollusca was one member, and the Dipleurula another member of this class.

If, however, we may draw conclusions from the representations of these ancestors given us in larval forms, *i.e.* in the larvae of Echinodermata on the one hand and in the Trochophore larvae on the other, there seems to have been a very great difference in the early development of these forms. The larvae of modern Ctenophora, and Annelida, and Mollusca agree in exhibiting in their development the very early segregation of organ-forming substances in distinct blastomeres, so that by the time the 4-cell stage is reached a single blastomere will only produce a quarter embryo. In a word, they exhibit determinate development. In the eggs of Echinodermata, on the contrary, the separation of organ-forming substances from one another takes place at a much later period; indeed for a considerable period, in fact until these eggs have attained the blastula stage, any sufficiently large fragment of the developing egg will regenerate the whole. That is to say, the development is indeterminate. It is quite possible that this difference in the character of the early development distinguished the two ancestors from one another before anything like modern Ctenophora, Annelida, Mollusca, or Echinodermata had developed.

We are now faced with the problem as to what interpretation we are to place on the extraordinary metamorphosis of Echinodermata. In searching for this interpretation we must recollect that a sudden metamorphosis, like that of Echinoidea, in which all the adult organs are formed under a veil of larval skin and suddenly break forth into activity, is no safe guide to us. In reconstructing ancestral history it is an indispensable condition that there should be continuous and gradual change of function and structure. No animal ever went to bed, so to speak, with one set of habits and woke up in the morning with another.

Between, however, the larval free-swimming life and the adult slow-moving one, there intervenes, in the case of Asteroidea and Crinoidea, a stage when the animal is fixed by the apex of the prae-oral lobe which has become converted into a stalk. This fixed stage suggests a reason for the radial development of organs which is so marked a feature in the adult Echinoderm. Fixed animals often develop their external food-catching organs in a radial manner, so as to sweep the whole neighbourhood for food. But the radial arrangement of tentacles, as we learn from Coelenterata and Annelida and Polyzoa, is quite consistent with the retention of bilateral symmetry. It can only be described as an idiosyncrasy of Echinoderms that bilateral symmetry is unstable, and that, therefore, radial symmetry was arrived at by the overgrowth of the organs of the left side and the partial suppression of those of the right side. To this day the first sign of unhealthiness in an Echinoderm larva is an unequal development of the larval arms.

We may assume, then, that the ancestral Dipleurula took first to holding on with its prae-oral lobe for short periods, a habit which became permanent; and that then the tendency to inequality between the two hydrocoeles, which we may suppose to have been latent in the stock, became a positive advantage as a short cut to radial symmetry, and was encouraged. But when this stage had been reached, the study of ontogeny seems to indicate that the Echinoderm stock became split into two stems. *For in Crinoid development the stomodaeum, into which the tentacles project, becomes rotated backwards, so that it eventually occupies the posterior pole of the body and is directed upwards; but in Asteroid development the mouth is moved to the left, and the disc is then flexed on the stalk in such a way that the mouth looks downwards.*

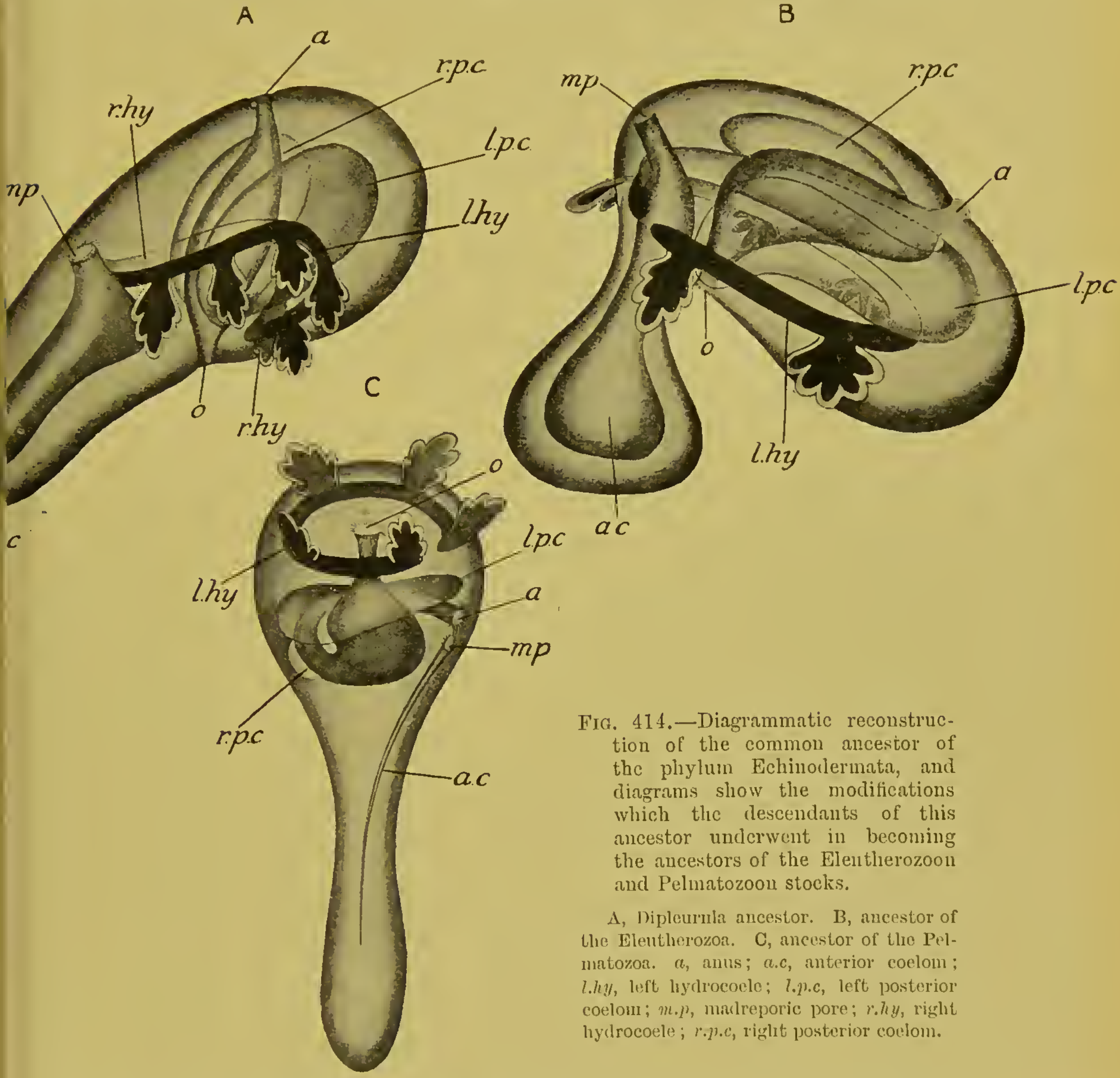

FIG. 414.—Diagrammatic reconstruction of the common ancestor of the phylum Echinodermata, and diagrams show the modifications which the descendants of this ancestor underwent in becoming the ancestors of the Eleutherozoon and Pelmatozoon stocks.

A, Dipleurula ancestor. B, ancestor of the Eleutherozoa. C, ancestor of the Pelmatozoa. *a*, anus; *a.c*, anterior coelom; *l.hy*, left hydrocoele; *l.p.c*, left posterior coelom; *m.p*, madreporic pore; *r.hy*, right hydrocoele; *r.p.c*, right posterior coelom.

As a consequence of this flexure, when the hydrocœle ring becomes completed by the meeting of its two ends, it encircles the base of the stalk in Asteroidea, but not in Crinoidea, where it is at the opposite end of the body from that occupied by the stalk.

It follows that the plates termed "radials" and "basals" in young Eleutherozoa and Pelmatozoa do not correspond to each other, but in each case must be looked on as a rearrangement of scattered plates when the whole body of the animal had been dominated by pentamerous symmetry.

Now the change of position which the mouth undergoes in the metamorphosing Crinoid is quite parallel with the change which it undergoes in the larva of a Tunicate, or of an Entoproct after fixation; and the reason in all three cases is the same, viz. the attempt to bring the mouth into a more favourable position for catching free-swimming prey which fill the water above it.

What then can have been the motive for the different shift of the mouth in an Asteroid? We can only surmise that it was an adaptation designed to bring the tentacles which surround the mouth into a more favourable position for grubbing in the mud and detritus on the substratum surrounding the animal. This supposition accords with the fundamental distinction which obtains at the present day between the habits of Eleutherozoa (Asteroidea, Ophiuroidea, Echinoidea, Holothuroidea), which in the majority of cases are scavengers, devouring dead animals and organic detritus lying on the bottom, and those of Pelmatozoa, which to this day feed on Plankton captured by currents produced by the cilia covering their tentacles.

The difficulty of imagining how a fixed animal could pick up a living by grubbing in the limited area of mud immediately surrounding it, is easily got over if we assume that this mud was not motionless, but *drifting*, if, in other words, the ancestral Asteroid had fixed itself in tide-ways, such as the gaps in coral reefs. Perhaps, indeed, the original purpose of fixation was to enable the animal to resist the pull of these tidal currents and avoid being swept helplessly along. When the old Asteroids extended their range into calmer water, then the breaking of the stalk would set them free to wander about and pick up a living under the new conditions.

The post-larval history of both Ophiuroids and Echinoids indicates that they are derived from the Asteroid stem. In both cases, after metamorphosis they creep about on their tube feet like an Asteroid, and in the case of the young Echinoid the terminations of the radial canals are free movable tentacles, and the dorsal surface, which later becomes the insignificant periproct, is larger than the ambulatory surface.

We can even form a guess as to what led to the evolution of these two orders out of a primitive Asteroid. The Ophiuroidea are merely Asteroidea in which the neuro-muscular system has attained a higher development. In this respect they are the "highest" Echinodermata; their movements are the most active, and their sensitiveness the most acute. The Echinoidea, on the contrary, seem to be a race of "climbing" Asteroidea. The typical regular urchin loves the vertical faces of stones and the crevices between stones. These give opportunities to employ the tube feet which spring from the upper surface of the body.

The origin of the Holothuroidea is most plausibly explained as a further development of primitive Echinoidea in the direction of haunting crevices. Just as the snake has lost its limbs in order to become adapted to wriggling through crevices, so the Holothuroid has lost its spines and reduced its plates to vestiges in order to render its body sufficiently flexible to worm its way through narrow openings. *Synapta* forms appropriately the end term in this series of modifications, for, in this form and its allies, wriggling through crevices has become changed into burrowing into sand and mud.

If the above interpretation of the developmental history of Echinodermata be accepted, and it may be fairly claimed to be in consonance with all the facts so far known, we may draw some interesting conclusions as to the modifications which the record of ancestral history, as embodied in ontogeny, has undergone. We see that a stage of development, viz. the fixed stage, may be completely omitted, as is the case in Ophiuroidea, Echinoidea, and Holothuroidea, and in this case the organ of fixation, the prae-oral lobe, is only vestigially developed; whilst, on the other hand, certain organs belonging to the preceding stage, viz. the ciliated band and its processes, are retained long after the period when, to judge from the stage of development of other organs, they should have disappeared. A precisely similar phenomenon is seen in the retention of the external gills of the salamander after all four limbs have become adapted for life on land. Further, an organ which should shift from one position to another by the unequal growth of surrounding parts, may disappear in one place and be reformed in another, as the mouth in Asteroidea and Echinoidea. It by no means follows that larvae which are primitive in one respect are primitive in all. Thus the Brachiolaria retains a stalk but forms a new mouth, whilst the Ophiopluteus has no trace of a stalk but retains the old mouth. Finally, the condition in which development culminates, viz. the adult condition, tends to be reflected back to earlier and earlier periods in ontogeny in the case of some organs, and this is what is termed **precocious development.**

From all these considerations it follows that different larvae which reflect in blurred form the same ancestral history, have become specifically modified; and this applies not only to larvae belonging to different classes, such as the Ophiopluteus and Echinopluteus, but to the larvae belonging to minor divisions within the class. Thus the larvae of Spatangoidea seem all to have the aboral process, whilst those of *Echinus* and its allies are totally devoid of it but possess ciliated epaulettes.

Lastly, what preserves a continuous record in ancestral history is the continuity of functional activity. If this be interrupted all sorts of aberrancies may occur. Thus the functionless larval gut of *Solaster* and *Antedon* is formed by a secondary development from the coelom, and the larval stomodaeum is either not formed (*Solaster*) or never reaches the gut (*Antedon*).

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CHAPTER XVII

PROTOCHORDATA

Classification adopted—

- **Hemichorda (Enteropneusta)**
  - **Balanoglossida**
  - **Cephalodiscida**
- **Cephalochorda**
- **Urochorda (Tunicata)**
  - **Larvacea**
  - **Ascidiacea**
    - **Ascidiae simplices**
    - **Ascidiae compositae**
    - **Ascidiae Luciae**
  - **Thaliacea**

Although the second and third volumes of this work are destined to treat of the embryology of the great group of Vertebrata or Chordata to which we ourselves belong, yet it is necessary to deal at the close of this first volume with the embryology of the lowest members of this group, for two reasons. First, because we must show how the Vertebrata are related to the Invertebrate groups whose embryology has been discussed in this volume; and, secondly, because one of the three groups which make up the division of Vertebrata known as Protochordata are still frequently regarded as Invertebrata.

The Vertebrate affinities of the Enteropneusta are denied by many zoologists, and although all now admit that the Urochorda are degenerate Vertebrata, yet these animals are so degenerate that their adult structure shows more similarity to that of a Podaxonian or Polyzoan than to that of any ordinary Vertebrate. The name Protochordata is, of course, nothing more than a convenient collective term for the poor relations of the vertebrate phylum which fall far below the rest of their brethren in structure and activity. It is by no means implied that its three subdivisions—Hemichorda, Cephalochorda, and Urochorda—are specially closely allied to one another. What indeed their relationship to one another actually is, will come out when their life-histories are studied.

We prefer the term Vertebrata to Chordata for the following reasons:—The word "Vertebrata" is so deeply established in the literature that it is impossible to eradicate it. If all Vertebrata must have true vertebrae, then not only must the groups classed as Protochordata be ejected from the phylum, but also Cyclostomata, Elasmobranchii, Dipnoi, Chondrosteid Ganoids amongst fish, and the lower Stegocephala amongst fossil Amphibia. If *Amphioxus* be "a-centrous" so also is the sturgeon. We must not draw a line which will bifurcate natural groups, hence the name "Vertebrata" must apply to the whole group of animals characterized by dorsal tubular nervous system, notochord, and gill-slits.

HEMICHORDA—ENTEROPNEUSTA

The Enteropneusta are represented at the present day by two totally distinct types of animal, both of which are marine. One, the Balanoglossida, resemble in outer appearance "worms," like Nemertea or Annelida, and lead a burrowing life, inhabiting the soft mud at the bottom of shallow waters all over the tropical and temperate regions of the world; whilst the other type, the Cephalodiscida, are sessile colonial animals, very much resembling Polyzoa in appearance and habit of life, and confined to the colder temperate, arctic, and antarctic regions of the sea. They were indeed, until very recently, confounded with Polyzoa.

About the embryology of the Cephalodiscida practically nothing is known; on one occasion when a fresh colony was dredged by a vessel proceeding on a voyage of Antarctic exploration, it was seen to emit oval ciliated larvae. The subsequent death of the naturalist attached to this vessel has prevented this most interesting discovery from being adequately followed up.

Of the development of the Balanoglossida, however, we know a great deal more. Long ago it was discovered by Metschnikoff (1870) that a remarkable larva named **Tornaria**, which had been frequently captured in the Plankton by the tow-net, and which had been mistaken for the larva of an Asteroid, was really the larva of a Balanoglossid worm. Then Bateson (1884–1885) worked out completely the life-history of a form, *Balanoglossus* (*Dolichoglossus*) *Kowalevskii*, which has somewhat yolky eggs and a shortened larval development. This development bears much the same relationship to the life-cycle which includes the Tornaria larva as does the development of *Asterina gibbosa* to that of *Asterias* with its bipinnaria larva.

Bateson's work, which is the foundation of our accurate knowledge of Enteropneust development, and which led to the view that the Enteropneusta have vertebrate affinities, was challenged by Spengel (1894), who questioned its accuracy and founded a view of the relationship of the Enteropneusta with Annelida on his own observations on Tornaria larvae. It is therefore important to notice that Bateson's conclusions have been confirmed, clarified, and reinforced by a series of researches executed by American zoologists, of whom the most noticeable are Morgan (1891, 1894), who worked out the later development of several varieties of Tornaria which occur on the east coast of North America; Ritter (1894), who described stages of the development of a species of Tornaria from the Pacific coast of North America; and Davis (1908), who described the development of a Balanoglossid (*Dolichoglossus pusillus*), with yolky eggs and shortened development, which appears to be closely allied to the species on which Bateson worked. Finally, a German, Heider (1909), has succeeded, for the first time, in obtaining the fertilized eggs of a Balanoglossid (*Balanoglossus clavigerus*), which has in its life-cycle a Tornaria larva; and he reared the eggs until the typical Tornaria larval form had been attained.

BALANOGLOSSUS

A fairly complete account of the development of a Balanoglossid can therefore be pieced together by adding Heider's work to that of Morgan. It is probable that the New England Tornaria belongs to some other species of the genus *Balanoglossus*. We prefer to take the researches of these two workers as the basis for our detailed account rather than to base our account on the development of *Dolichoglossus*, as worked out by Bateson and Davis, because we hold that a roundabout development, including the formation of a pelagic larva with a long free-swimming existence, is the primitive type of development, and that a shortened development with a larva having a very short free-swimming life, and in which the adult features appear very early, like that of *Dolichoglossus*, is a secondarily modified condition of affairs.

Heider obtained the fertilized eggs amongst a consignment of the adults which were collected at the Zoological Station at Trieste and sent to him at Innsbrück. During the journey males and females discharged their genital products and a natural fertilization (see p. 485) resulted. Subsequently, when on their arrival the adults were placed in sea-water with sand at the bottom, they formed burrows in it, and at the mouth of one of the burrows a slimy mass containing thousands of eggs was seen. These eggs, however, were not fertilized.

The eggs were small and filled with coarse grains of yolk which were uniformly distributed, so that the only way of distinguishing animal and vegetative poles was the nearness of the egg-nucleus to the former. In the fertilized eggs the usual two polar bodies were formed, of which the first divided.

The eggs segmented with perfect regularity into blastomeres of equal size, and gave rise to perfectly spherical thick-walled **blastulae**, with comparatively small **blastocoeles** whose walls were composed of a very large number of narrow, cylindrical, ciliated cells. Heider considered that the development up to this point recalled that of Echinoidea, but the Balanoglossid blastula differs from the Echinoid blastula in having thicker walls. Further, it is composed of taller cells than the latter blastula. In both cases, however, it is obvious that we have to do with indeterminate cleavage; nothing even distantly suggesting the spiral cleavage of Annelida is to be seen.

The blastula stage had been already attained by the vigorous eggs when the material came into Heider's hands. On the following day the blastulae became hemispherical, by the flattening of one side, as do Echinoid blastulae, and the **gastrula** stage was attained by the invagination of this flattened surface. The archenteron did not completely fill up the blastocoele, since, *coincidently with its formation, the embryo grew in length.* The **blastopore** was at first wide, but became reduced to a narrow pore and finally closed altogether; but the endodermic sac remained connected with the ectoderm at the spot where the closure took place, and here, at a slightly later stage, the **anus** was formed; so that we may say that in *Balanoglossus clavigerus* the blastopore becomes the anus, but for a brief period it is closed.

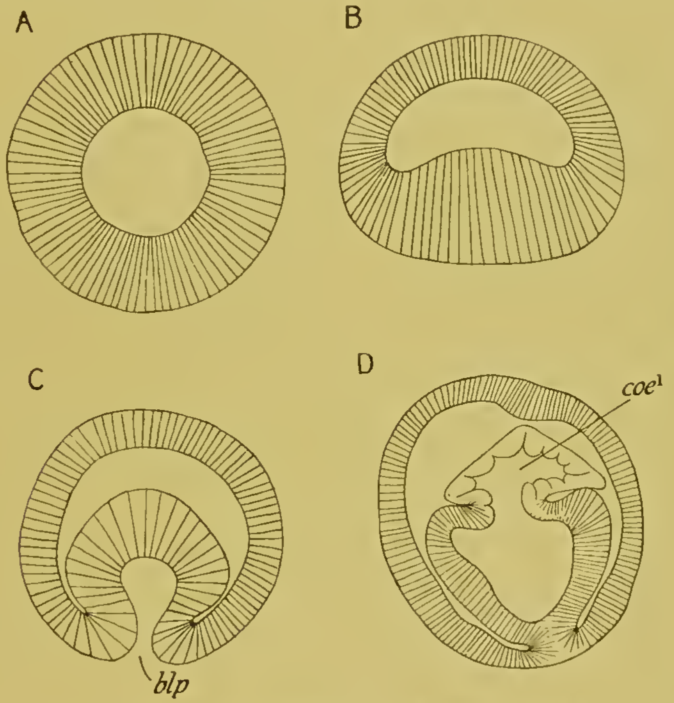

FIG. 415.—Early stages in the development of *Balanoglossus clavigerus*. (After Heider.)

A, Blastula (about one day old). B, incipient gastrulation (one and a half days old). C, gastrula (two days old). D, formation of the anterior coelom. *blp*, blastopore; *coe*¹, anterior coelom.

The front end of the somewhat elongated embryo then broadened out, and the whole organism became flattened so as to have broad dorsal and ventral surfaces and narrow sides. The front portion of the archenteron became separated from the rest by a groove, and so constituted the **anterior coelomic vesicle**, the rudiment of the **proboscis coelomic cavity** of the adult (Fig. 415). The cells forming the wall of this sac lost their cylindrical form and became flattened, and sent out pseudopodia by which the coelomic sac was anchored, so to speak, to the ectodermal walls of the body.

The anterior coelom now sent out a prolongation which reached the anterior pole of the embryo; at this point an **apical plate** was

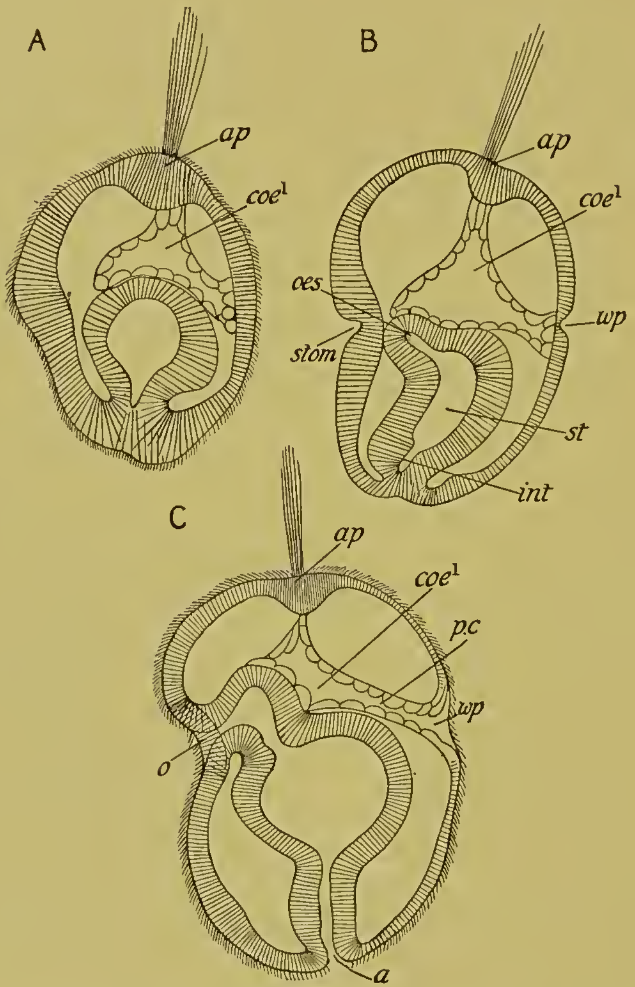

FIG. 416.—Later stages in the development of *Balanoglossus clavigerus*. (After Heider.)

A, formation of the apical plate. B, formation of mouth and of pore-canal. C, mouth anus and water pore formed. *a*, anus; *ap*, apical plate; *coe*¹, anterior coelom; *int*, intestine; *o*, mouth: *oes*, endodermal section of oesophagus; *p.c*, pore-canal; *st*, stomach; *stom*, stomodaeum; *w.p*, water-pore.

formed by a thickening of the ectoderm, and the cells forming this thickening developed long stiff cilia, so that in this way a typical apical plate was formed. From the coelomic sac also a dorsal projection was developed which grew backwards and fused with the ectoderm, and here, somewhat later, an opening was effected which became the **proboscis-pore** or **water-pore** of the adult (*w.p*, Fig. 416, C).

The front end of the gut, as we may term the remnant of the archenteron, now became bent towards the ventral surface, and here came into contact with the ectoderm; this ventral prolongation constitutes the rudiment of the larval **oesophagus**. At the same time the hinder part of the gut exhibited the constriction separating a globular **stomach** from a narrow **intestine**.

At this stage, reached one and a half days after the blastula stage had been attained, the embryo burst the egg-membrane and began its career as a free-swimming larva which was uniformly ciliated all over. On the next day both mouth and anus broke through; there was a very shallow wide **stomodaeum** similar to but much shallower than the stomodaeum of the Echinoderm larva, but almost all the oesophagus was of endodermal origin. Oesophagus, stomach, and intestine were now sharply marked off from one another, and the whole interior of the alimentary canal was ciliated. The anterior prolongation of the coelomic sac became solid and so formed the **apical string** (*ap.s*, Fig. 417, B), which connects the apical plate with the proboscis-coelom and with the oesophagus, and on the next day its cells developed contractile fibrils, and the string was thus converted into a muscular strand. From this string were also given off the first **mesenchyme** cells which wander into the blastocoele. These consequently originate at a later stage of development in this larva than in any Echinoderm larva studied. Where the posterior aspect of the vesicle touched the oesophagus, pseudopodium-like strings grew out from its cells which were converted into circular muscles (*musc*, Fig. 417, A), and we may remind our readers that the circular muscles of the oesophagus of the Echinopluteus larva are formed in a precisely similar way.

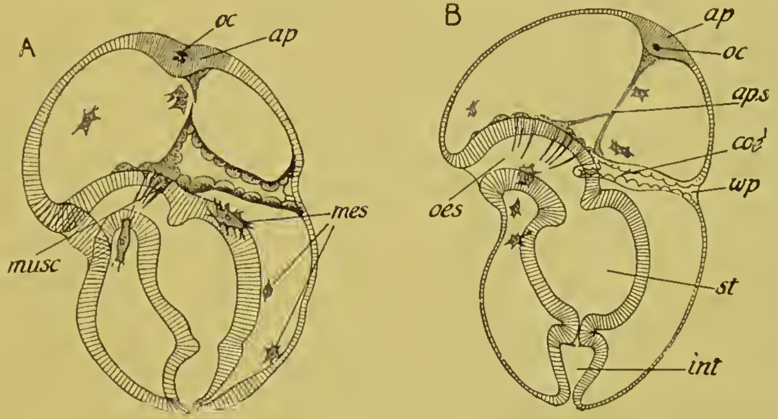

FIG. 417.—Still later stages in the development of *Balanoglossus clavigerus*. (After Heider.)

A, formation of mesenchyme. B, retreat of anterior coelom from apical plate, and formation of apical string. *ap*, apical plate; *ap.s*, apical string; *coe*^{1}, anterior coelom; *int*, intestine; *mes*, mesenchyme cells; *musc*, muscular fibrils—outgrowths of mesenchyme cells; *oc*, eye-spot; *oes*, oesophagus; *st*, stomach; *w.p*, water-pore.

Heider was able to keep the larvae living for eight days. Though still ciliated all over, a concentration of ciliated cells was observable along certain lines corresponding to the position of the ciliated bands of the full-grown Tornaria larva; these are a **longitudinal folded band** with a marked backwardly-directed prae-oral loop, in all respects similar to the main ciliated band of a young Bipinnaria larva, and a posterior transverse ciliated band which is the main external feature which distinguishes the Tornaria larva from the young Bipinnaria and Auricularia larvae. This band corresponds roughly in position to the telotroch of Annelid larvae and will receive the same name, viz. **telotroch** (*ttr*, Fig. 418). The apical plate had by this time developed two **eye-spots** which were simple cups of ectoderm cells surrounded by pigment. The plate is situated just at the spot where the prae-oral loop originates from the main part of the longitudinal ciliated band. The anterior coelom now sent out two posterior spurs which arched round the oesophagus, and in the oldest larvae Heider was able to detect the origin of a pair of posterior coelomic vesicles which form the rudiment of the **trunk-coelom.** These arose as evaginations, with slit-like lumina, of the anterior wall of the intestine, just behind the groove which marks it off from the stomach.

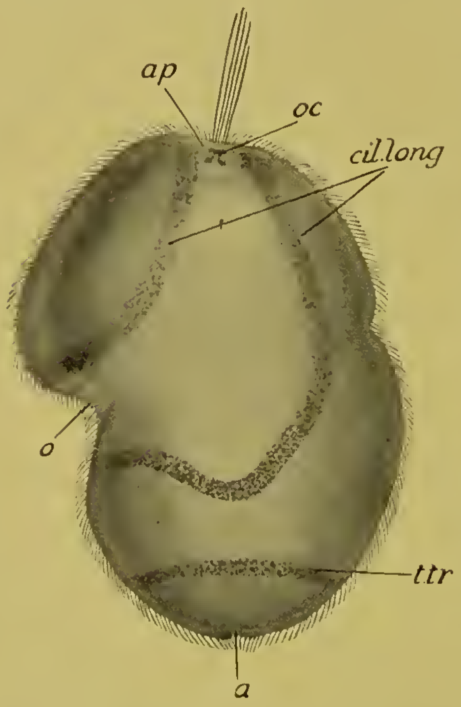

FIG. 418.—Surface view of the young Tornaria larva of *Balanoglossus clavigerus*, four days old. (After Heider.)

a, anus; *ap*, apical plate; *cil.long*, longitudinal band of cilia; *o*, mouth; *oc*, eye-spots; *ttr*, telotroch, i.e. posterior transverse band of cilia.

Morgan's observations (1891) on the development of the New England Tornaria commence just at the stage where Heider's observations leave off. His material consisted of a swarm of Tornaria larvae of all ages which were caught by the tow-net off Wood's Hole, Massachusetts, in the summer of 1890. The youngest of these were only $\frac{1}{4}$ mm. long, but in them the anterior coelom was entirely separated from the gut and the proboscis-pore had been formed. The two ciliated bands were distinct, but the anterior one was barely folded. The posterior coelomic sacs had not yet been formed. The apical plate was completely fused with the sides of the longitudinal band at the prae-oral pole of the larva, where these sides approach most closely to one another; it thus formed a bridge delimiting a prae-oral loop from the rest of the band. On the apical plate were two eye-cups, an anterior and posterior. These were hemispherical pockets of clear cells, each cell terminating in a conical spike. Between the two eye-cups were a mass of pigmented cells. At the base of the apical plate a mass of nervous fibrils could be seen (Fig. 419).

In somewhat older larvae a solid mass of cells could be seen lying in the blastocoele, above and somewhat to the right of the proboscis-pore. These cells, whose origin Morgan could not determine, we conclude, from the development of *Dolichoglossus*, have been derived from the posterior wall of the anterior coelom. They are the rudiment of the **pericardium**, for in a slightly older larvae they became hollowed out to form a vesicle (*per*, Fig. 421). Between this vesicle and the oesophagus and the posterior wall of the anterior coelom there existed a V-shaped space, filled with blastocoelic fluid and opening into the blastocoele behind. This space is the rudiment of the peculiar dorsal **heart** of Balanoglossida, which has been seen to pulsate.

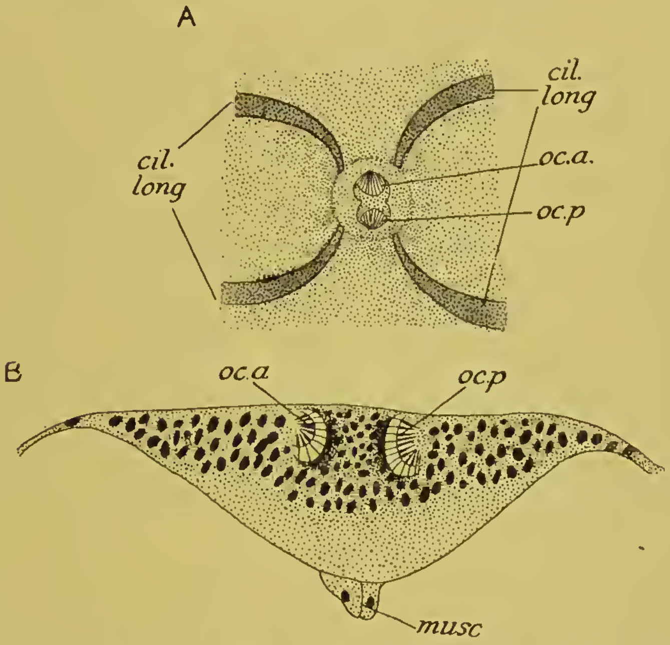

Fig. 419.—Illustrating the structure of the apical plate and eyes of a full-grown New England Tornaria larva. (After Morgan.)

A, apical view of full-grown Tornaria, showing the apical plate and eyes, and the relation of the plate to the longitudinal ciliated band. B, antero-posterior longitudinal section through the apical plate and eyes. *cil.long*, longitudinal ciliated band; *musc*, muscle cells belonging to the apical string; *oc.a*, anterior eye; *oc.p*, posterior eye.

In larvae older than the second stage described, the posterior or **trunk coelomic cavities** could be seen developing exactly as Heider described in the case of *Balanoglossus clavigerus*. Slightly later, the middle or **collar coelomic cavities** originated as solid evaginations of the posterior part of the wall of the stomach (Fig. 420). Both pairs of rudiments, after being cut off from the alimentary canal, formed, for a time, little solid disc-like bodies applied to the sides of the stomach and intestine. Only very gradually did they acquire distinct lumina, and extend dorsally and ventrally so as to encircle the gut and meet their fellows in the mid-dorsal line. Their extension in an antero-posterior direction was very restricted until the very close of larval life.

When we examine the structure of the full-grown Tornaria larva before metamorphosis sets in, we find that the longitudinal band of cilia, in addition to becoming differentiated into prae-oral and post-oral loops, has been thrown into several secondary folds which we may term arms. If we applied the nomenclature which Mortensen has invented for Echinoderm larva (see p. 464), we should say that the longitudinal band, in addition to giving off a backwardly directed **prae-oral loop,** gave off also a forwardly directed **anal loop.** Where the anal loop is given off there is, on each side, a conspicuous **postero-lateral arm** of the band. From the sides of the prae-oral loop a large **prae-oral arm** is given off, and on the main portion of the band, just behind this loop and behind the apical plate, is also to be found a backwardly directed **antero-dorsal arm**; on the left side is the proboscis-pore.

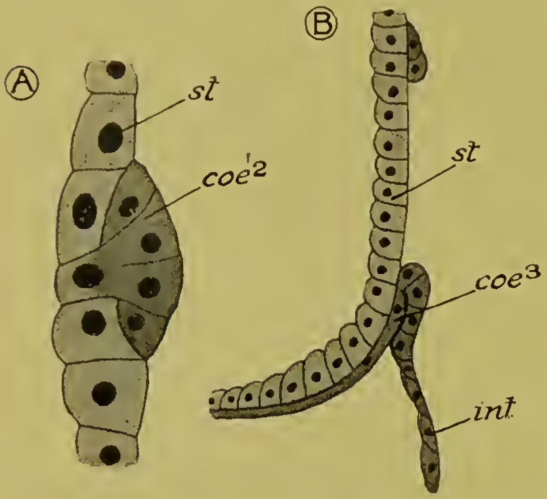

FIG. 420.—Illustrating the origin of the middle and posterior coelomic vesicles in the New England Tornaria. (After Morgan.)

A, longitudinal section through the anterior portion of the wall of the stomach, in order to show the origin of the collar cavity. B, longitudinal section through the posterior portion of the wall of the stomach, and through the wall of the intestine, in order to show the origin of the trunk-cavity. *coe*², rudiment of collar-cavity; *coe*³, rudiment of trunk-cavity; *int*, intestinal wall; *st*, stomach wall.

The ventral ectoderm forming the oral field, included between prae-oral and anal loops, is thin and flat. Outside this area it is composed of cubical cells, and it becomes quite thick on the anterior part of the larva around the apical plate, and to a lesser extent around the anus within the area included within the posterior ciliated band. The epithelium lining the dorsal and ventral walls of the oesophagus is ciliated; the dorsal cilia stop short of the stomach, but the ventral ciliated band is continued into the general ciliation of the walls of the stomach.

The sides of the oesophagus are produced into several pairs of pockets, these are the rudiments of the future **gill pouches** (*g.p*, Fig. 421). It will be particularly noted that in the Tornaria larva the gill region is far in front of the middle or collar body-cavity which embraced the hinder end of the stomach, whereas in the adult, the gill region is behind the collar-cavity altogether. The stomach and intestine are separated by a diaphragm perforated by a hole, round which is a wisp of long cilia which beat so as to produce rotatory movements.

As metamorphosis draws on the larva decreases in size and becomes more opaque. These changes are due to a diminution in size of the blastocoele and to a change in shape of the ectoderm cells, which become more columnar. The longitudinal band of cilia becomes indistinct, except along its posterior border; the circular band remains active, but it becomes shifted farther back by a growth of the region of the body intervening between it and the longitudinal band.

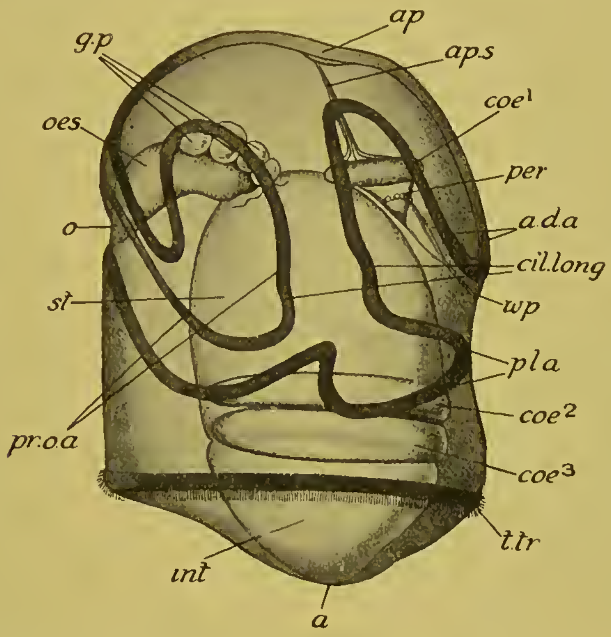

FIG. 421.—Full-grown New England Tornaria seen from the left side. (After Morgan.)

a, anus; *a.d.a*, anterior dorsal arm of the longitudinal ciliated band; *ap*, apical plate; *ap.s*, apical string; *cil.long*, longitudinal ciliated band; coe^1, anterior (proboscis) coelom; coe^2, middle (collar) coelom; coe^3, posterior (trunk) coelom; *g.p*, gill pouches; *int*, intestine; *o*, mouth; *oes*, oesophagus; *per*, pericardial sac; *p.l.a*, posterior lateral arm of the longitudinal ciliated band; *pr.o.a*, prae-oral arm of the longitudinal ciliated band; *st*, stomach; *t.tr*, telotroch; *w.p*, water-pore.

The larva now drops to the bottom and glides over it by the help of the cilia of the circular band. The anterior region of the body grows in length and becomes conical, and gradually takes on the shape of the **proboscis** of the adult. The posterior wall of the anterior coelom or **proboscis-cavity** becomes curved forwards and thrown into a number of folds, and this constitutes the excretory tissue or **head kidney** of the adult (*ex*, Fig. 422, A and B). The posterior border of the longitudinal band becomes slightly invaginated and marks the front edge of the **collar region**; the hinder limit of this region is marked by a new transverse groove, which now appears on each side and passes forwards near the mid-dorsal line to join the groove marking the front border of the collar. In the mid-dorsal line there is consequently a strip of ectoderm uncrossed by the grooves.

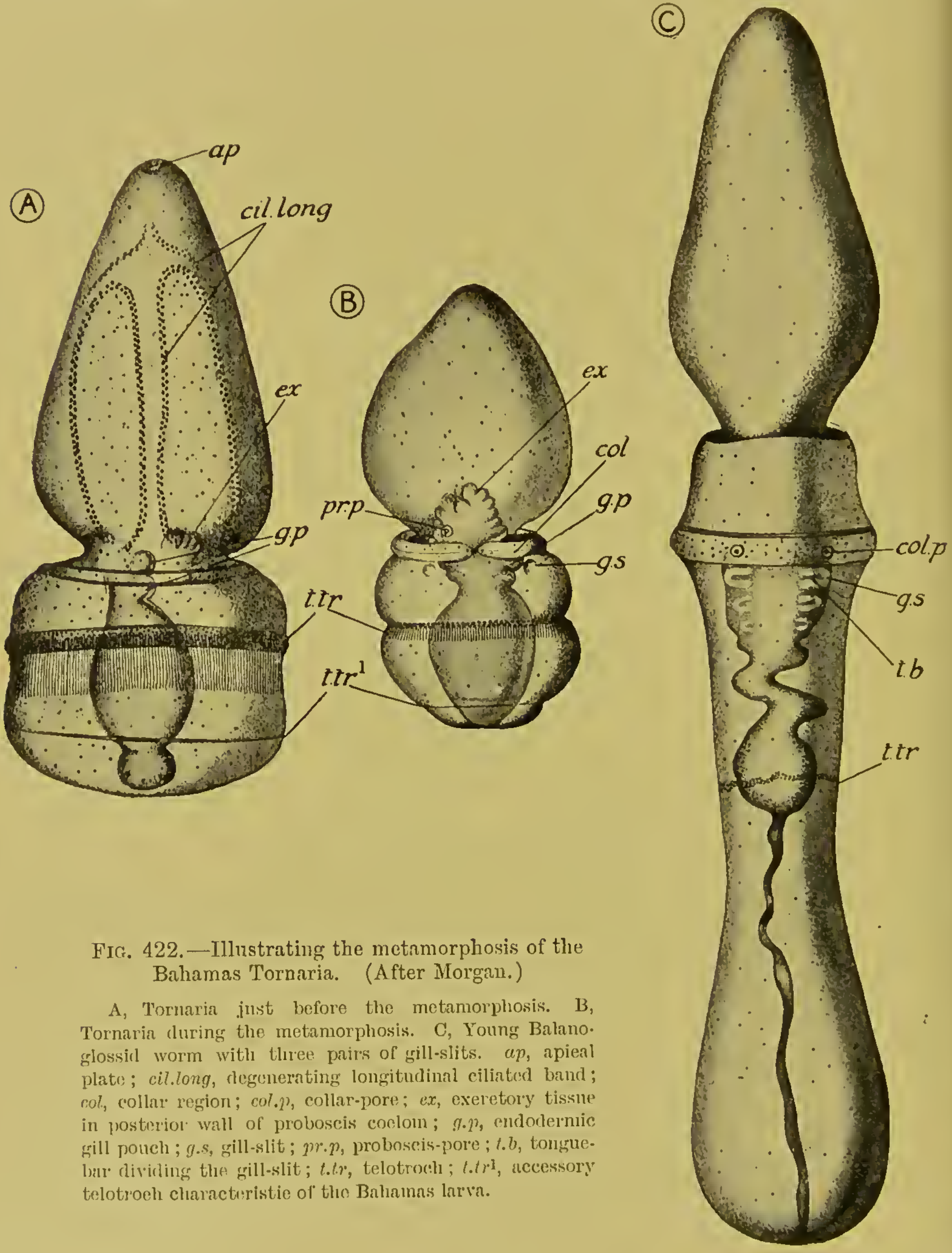

Fig. 422.—Illustrating the metamorphosis of the Bahamas Tornaria. (After Morgan.)

A, Tornaria just before the metamorphosis. B, Tornaria during the metamorphosis. C, Young Balanoglossid worm with three pairs of gill-slits. *ap*, apical plate; *cil.long*, degenerating longitudinal ciliated band; *col*, collar region; *col.p*, collar-pore; *ex*, excretory tissue in posterior wall of proboscis coelom; *g.p*, endodermic gill pouch; *g.s*, gill-slit; *pr.p*, proboscis-pore; *t.b*, tongue-bar dividing the gill-slit; *t.tr*, telotroch; *t.tr*^{1}, accessory telotroch characteristic of the Bahamas larva.

This is the rudiment of the dorsal **neural plate.** It becomes depressed beneath the surface, flaps of the adjacent ectoderm, which we may term **neural folds,** meet over it, and in this way a dorsal **neural tube** is formed (*n.t.*, Fig. 423).

About this time, from the anterior end of the oesophagus, a median dorsal, forwardly-directed pouch grows out; this is the rudiment of the **notochord** (Fig. 424). Transverse sections show that this pouch is continued backwards as a dorsal section of the oesophagus, separated by lateral grooves from the rest, and that in these grooves lie two chitinous rods secreted by oesophageal epithelium. These are the legs of the **collar skeleton,** which is merely a specially thickened portion of the cuticle secreted by the bases of the notochordal cells, and corresponds to the primary cuticular sheath of the notochord of higher Vertebrata. The gill pouches become applied to the ectoderm, and here the external gill openings are formed. **Tongue bars,** *i.e.* vertical folds of the dorsal walls of these pouches, dividing these cavities almost into two, are formed before the external openings appear (*t.b,* Fig. 422, C).

The larva has now almost assumed the form of the adult, but a remarkable change in the dimensions of the gut has yet to make its appearance. This has been described as a "pulling in" of the anterior portion of the gut; but it might be more aptly described as a lengthening of the region in front of the gills. As a consequence the gills become pushed back till they lie behind the collar region, the diaphragm separating stomach and intestine disappears, and these two regions are consequently no longer distinguishable.

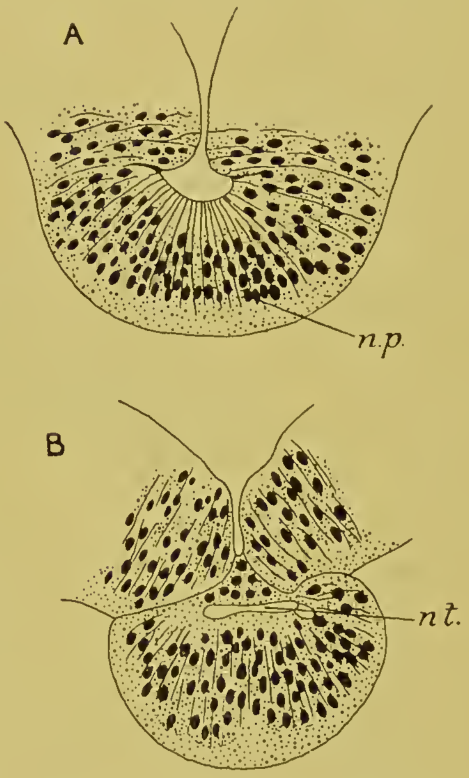

Fig. 423.—Illustrating the development of the dorsal nervous system in the metamorphosing Bahamas Tornaria. (After Morgan.)

A, cross-section through the nervous system of a younger specimen—the nerve plate is flanked by two ectodermic folds. B, cross-section through the nervous system of an older specimen—the ectodermic folds have met above the nerve plate and the nerve plate has become a nerve tube. *n.p,* nerve plate; *n.t,* nerve tube.

Morgan was able to keep his oldest Tornaria larvae alive for three days after they had been caught, and to watch them metamorphose as has been described, but he did not see them begin to burrow. Subsequently he visited the Bahamas and found there, in the Plankton, two much larger varieties of Tornaria, the metamorphosis of one of which (the Nassau Tornaria) he was able to study in some detail. His results are recorded in a second paper (1894).

This larva differs from the New England one, not only in its much greater size, but in the elaboration of the processes of its longitudinal ciliated band. The sides of the prae-oral loop and of its outgrowths, the prae-oral processes, as well as the sides of the main portion of the longitudinal ciliated band and of its outgrowths, the antero-dorsal processes, are fringed with small, secondary, finger-like processes. There is also a second circular band of cilia behind the telotroch, but much more feebly developed. According to Morgan, in this larva the collar-cavities originate by the aggregation of scattered mesenchyme cells. This statement is in the highest degree improbable; such an impression might be produced on the mind of an observer if the critical stages of the development were missed out.

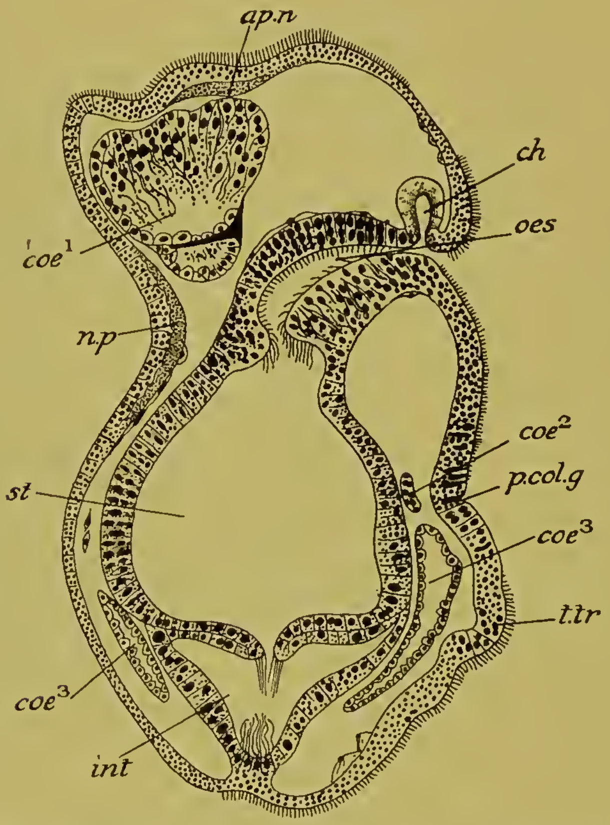

Fig. 424.—Longitudinal sagittal section through the New England Tornaria immediately after its metamorphosis into the Balanoglossid worm. (After Morgan.)

ap.n, nervous tissue underlying the apical plate; *ch*, rudiment of notochord; *coe*^{1}, proboscis coelom; *coe*^{2}, collar coelom; *coe*^{3}, trunk coelom; *int*, intestine; *n.p*, dorsal neural plate in the collar region; *oes*, oesophagus; *p.col.g*, posterior collar groove; *st*, stomach; *t.tr*, telotroch.

The principal additional points which Morgan made out satisfactorily in these larvae were, the formation of the **collar pores**, and the first traces of the **genital organs**. The **collar pores** originate as two ectodermal invaginations situated on the sides of the body, not far from the mid-dorsal line at the hinder region of the collar. The tips of these invaginations become fused with the collar coelom on either side, and here the collar pores are formed. The first and second pairs of gill pockets also open into these invaginations, which Morgan, following Bateson, compares to the atrial cavity of *Amphioxus*.

The **genital organs**, which in Balanoglossida are very small and numerous and widely scattered, and of which each possesses its own duct, originate as local proliferations of the cells forming the outer walls of the trunk coelom (*gon*, Fig. 425).

The thickened band of ectoderm cells, marking the site of the posterior ciliated band, persists for a long time in the Bahama larva; and it is possible to make out that the trunk of the young Balanoglossid worm is made up, in about equal proportions, of the regions in front of and behind this band, in both of which very great growth in length takes place. Morgan was able to see the young metamorphosed forms burrowing into the mud at the bottom of his culture vessels.

The Tornaria found by Ritter (1894) on the Pacific coast of North America, agrees with the Bahama form in having secondary processes (tentacles) developed along the course of the prae-oral processes, and also on the main part of the longitudinal band, but these processes are fewer in number and blunter than in the Bahama larva. There is also an additional pair of processes on the horizontal part of the longitudinal band, situated near the mid-ventral line which, following Mortensens' notation, we may name **post-oral**. As in the Bahama larva there is a second transverse band of cilia situated behind the first main one.

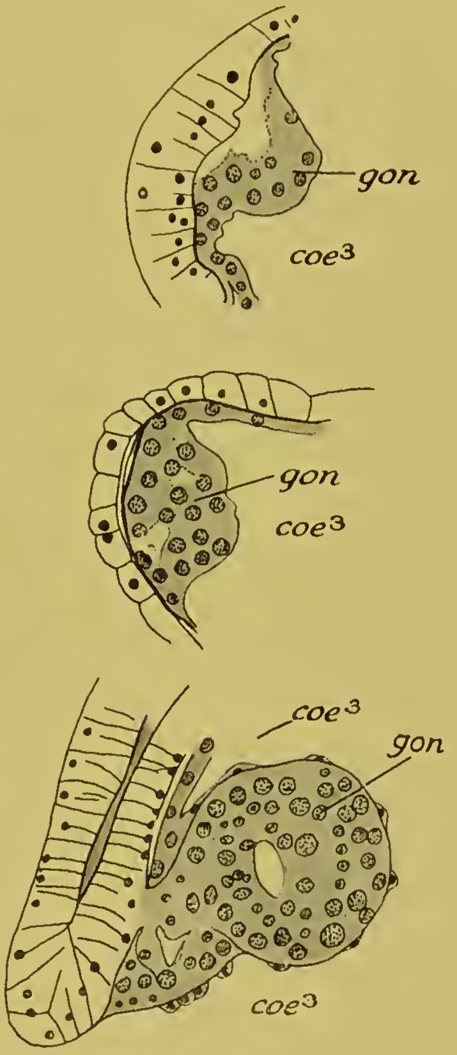

Fig. 425.—Portions of transverse sections through the trunk region of three young Balanoglossid worms of different ages, in order to illustrate the development of the genital organ in the Bahamas species. (After Morgan.)

coe^3, trunk-cavity; *gon*, developing genital organ.

In this Tornaria, in the mid-ventral wall of the oesophagus there is a thick ridge carrying especially long cilia. This ridge is compared by Ritter to the **endostyle** of *Amphioxus*; it is apparently continuous with a lesser ridge of the same kind in the wall of the stomach. The nerve plate is converted into a nerve tube in exactly the same manner as in the New England Tornaria.

DOLICHOGLOSSUS

We must now devote some consideration to the shortened development of *Dolichoglossus* as worked out by Bateson and Davis. Bateson (1884–1885) found that the eggs of the species he worked at (*D. kowalevskii*), were shed into the soft mud which the parent worm inhabited, and there passed through the whole of their development.

The eggs were comparatively large (about ·4 mm. in diameter). filled with a yellowish yolk and provided with a firm egg-shell. Attempts at artificial fertilization led only to abnormal segmentation and death of the embryo, and so all stages of development had to be procured from the mud, and Bateson's method of finding them was not a little ingenious. He procured a quantity of mud in which the adults lived, and to this was added a number of adult worms cut into small fragments; the whole mixture was stirred up with sea-water, avoiding rotatory currents. Then, after waiting a minute or two to allow the agitation to cease, the upper layers of the fluid were siphoned off until the layer containing the fragments of the adult worm was reached. This layer was then siphoned off and carefully preserved. In it were found the fertilized eggs and the embryos and larvae in all stages of their development, since all these were about the same specific gravity as the fragments of the parent worm.

The embryos in their early stages of development agree precisely with the embryos of *Balanoglossus clavigerus* as described by Heider. The proboscis coelom is formed as in the Tornaria larva, but the collar and trunk coelomic cavities arise about the same time as hollow evaginations of the gut. When the larva escapes from the egg-membrane, the same stage of development has been attained as is reached by the Tornaria larva just before its metamorphosis. The larva possesses an apical tuft of cilia, and a ciliated band encircling the posterior region of the body, evidently homologous with the telotroch in the Tornaria larva. The whole ectoderm is beset with short cilia, but there is no trace of the characteristic folded, longitudinal ciliated band of the Tornaria larva. Two transverse grooves, including between them a narrow transverse ridge, have appeared. This ridge is the rudiment of the collar region. Some distance behind this the first gill-pore appears on either side; but the collar region grows back till it covers not only this gill-slit, but also the second which is subsequently formed.

The changes necessary to reach the adult condition are few; the apical tuft and posterior ciliated band disappear, the prae-oral portion of the larva grows in length and becomes conical, the gill-slits increase in number, and the trunk region grows in length.

The pericardial vesicle originates as a solid outgrowth of the posterior horn of the proboscis-cavity; and the dorsal nerve cord is delaminated as a solid strip of ectoderm, into which canals extend subsequently from the anterior and posterior ends of the collar where it remains in connection with the ectoderm.

Davis' account of the embryology of *D. pusillus* (1908) agrees in many points with Bateson's account of the development of *D. kowalevskii*, which we have just summarized. The embryo hatches out and begins free life when the collar region is delimited and one pair of gill-pores has appeared.

Owing to the fact that the mud was of a more cohesive kind than that in which *D. kowalevskii* lives, it was possible, at low tide, to take spadefuls of mud containing the burrows intact, and when these burrows were broken open the fertilized eggs were seen clinging to one side of them. A mixture of corrosive sublimate and acetic acid, osmic acid, and Lo Bianco's chrom-osmic mixture, were used for preserving the embryos. The embryos and larvae, which were removed from the burrows, lived and completed their development in vessels of clean water in the laboratory.

When the larvae are first hatched they swim in spirals and rise to the surface, but soon tire and drop to the bottom (Fig. 426). These swimming efforts at first occur at regular intervals, but the intervals become gradually longer and longer, and finally the larvae only glide over the bottom. Soon muscular movements are observable in the proboscis, and with the loss of cilia the burrowing life of the adult is begun. These spasmodic upward movements, however, give opportunities to the tidal current to waft the larvae far from their birthplace.

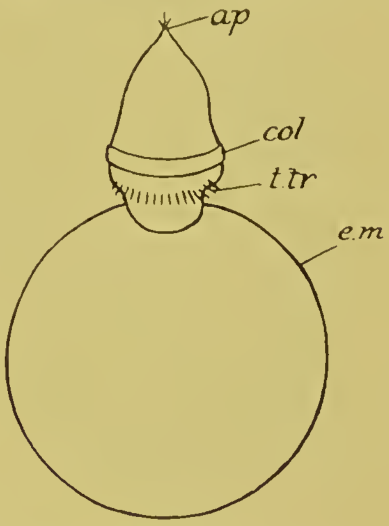

Fig. 426.—The larva of *Dolichoglossus pusillus* in the act of escaping from the egg-membrane. (After Davis.)

ap, apical plate; *col*, collar region; *e.m*, egg-membrane; *t.tr*, telotroch.

The principal points of interest in the development of *D. pusillus*, as brought out by Davis, are as follows: (1) The cleavage is not quite regular, for, as in the egg of *Amphioxus*, the upper four blastomeres of the 8-cell stage are smaller than the lower four, and there is a cleavage pore; that is to say, that the incipient blastocoele, formed by the separation of the blastomeres, opens above and below. (This also occurs in the egg of *Amphioxus*.) (2) Both middle and posterior pairs of coelomic cavities arise as outgrowths of the anterior body-cavity, so that, as in Echinoderms, there is one anterior evagination of the archenteron which gives rise to all the body-cavities (Fig. 427).

AFFINITIES OF THE ENTEROPNEUSTA.

The question of the vertebrate affinities of the Enteropneusta stands or falls with the homology of the nerve cord, notochord, and gill-slits with the similarly-named structures in the higher vertebrates. Our own view, that the two sets of structures are really homologous, and that Enteropneusta are a degenerate offshoot from the base of the vertebrate stem, will be supported by evidence given when the development of the Cephalochorda is discussed, since these are the lowest forms admitted by all to be true Vertebrata.

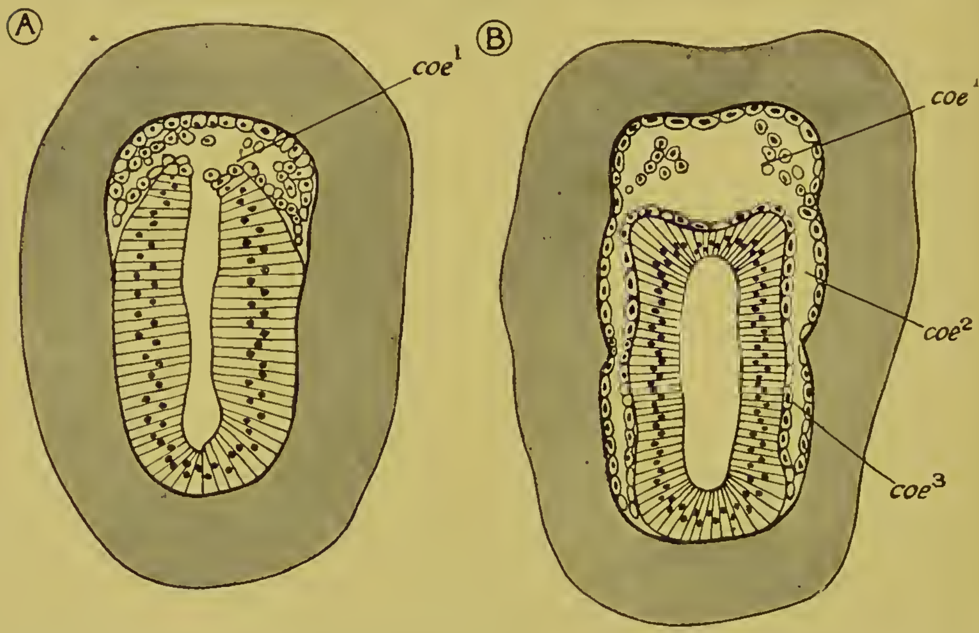

FIG. 427.—Longitudinal frontal sections through two embryos of *Dolichoglossus pusillus* in order to illustrate the development of the body-cavities. (After Davis.)

A, through younger embryo—the proboscis coelom is arising as an anterior evagination of the gut. B, through older embryo—the collar coelom and trunk coelom are arising by the transverse division of posterior-directed tongues of the anterior coelom. *coe*¹, the proboscis coelom; *coe*², the collar coelom; *coe*³, the trunk coelom.

The development of *Balanoglossus* and *Dolichoglossus* is, however, calculated to throw light on the previous history of the common stock of Vertebrata and Enteropneusta; it takes us, as Lankester has well said, into prechordal times. It will be noted that the description of the development of *Dolichoglossus*, given by Bateson, is confirmed in all important points by the description of the development of *Balanoglossus* as given by Heider and Morgan: we may therefore take it as being thoroughly well established. Spengel's criticisms (1894) of Bateson's results, based on his own observations on Tornaria larvae, made many years ago by methods which are now superseded, are no longer valid, as he himself would be the first to admit.

We may therefore note that the Tornaria larva bears, in many respects, a strong resemblance to the Bipinnaria larva of Asteroidea, and, in external appearance, to the Auricularia larva of Holothuroidea; from both of which it differs principally in possessing the posterior ciliated band and the well-developed apical plate. The apical plate, however, turns up in the Crinoid larva and in the late Echinopluteus larva, and it was probably once a feature of all Echinoderm larvae. The tendency for the longitudinal ciliated band to rearrange itself in transverse ciliated bands is exemplified by the late Echinopluteus larva, by the "Pupa" of Holothuroidea, and by the Crinoid larva. The posterior ciliated band of the Tornaria larva may be the result of such a rearrangement. We arrive, finally, at the conclusion that no important difference divides the Tornaria from the Echinoderm type of larva; *the great difference between the two lies in the nature of their metamorphoses.*

It follows that Echinodermata and Enteropneusta (and through the latter the whole of the Vertebrata) are descended from the same stock of simple free-swimming animals. In this ancestral stock the coelom was already divided into three sections on each side, such as become delimited in the coelom of the Echinoderm larva as growth proceeds; and the middle sections, called **hydrocoeles** in Echinodermata, and **collar-cavities** in Enteropneusta, were produced into ciliated tentacles.

The main stem of the common stock which, in its day, must have constituted a dominant type of animal, kept to the sea and gave rise to the higher Vertebrata; one division of which, the Pisces, still dominate that element. An offshoot, which was destined to give rise to both Echinodermata and Enteropneusta, dropped to the bottom and took to gliding over the soft ooze. Certain of these gliders finally fixed themselves to the substratum by the **prae-oral lobe**, and gave rise to the Echinodermata; whilst others degenerated into burrowing habits and became Balanoglossida. But still others seem to have learned to fix themselves by the **ventral integument**, and thus gave rise to the other division of Enteropneusta known as the Cephalodiscida. In these last, as in the Echinodermata, the middle or collar body-cavities are prolonged into ciliated tentacles; in fact, the great difference between them and the Echinodermata (apart from the part of the body with which they fix themselves) is that in the Cephalodiscida both collar-cavities are equally developed, whereas in Echinodermata the left overpowers the right, and leads to that peculiar ring-shaped growth of the left hydrocoele which later imposes a radial symmetry on the primitive bilateral symmetry. It is quite conceivable that Brachiopoda also, as indicated by the three segments of their larva, may be distantly related to the same type, but the substantiation of this suggestion would require a great deal of further work.

If the reasoning outlined above be sound, a most interesting conclusion can be drawn as to the origin of the peculiar Vertebrate nervous system, which differs so profoundly from the central nervous systems of most invertebrates. The central nervous system of Vertebrata must have been originally only a local intensification of the general plexus of nerve fibres underlying the skin, which exists both in Echinodermata and Enteropneusta. Its original function was probably to act as a co-ordinating centre for the activities of the tentacles of the two hydrocoels. The importance which it acquired in this way was retained when these tentacles were lost, and it became the dominating nervous centre for all the organs of the animal.

CEPHALOCHORDA

The Cephalochorda consist of a number of closely-allied species of sand-inhabiting animals which are found in the tropical and warmer temperate regions of the world. They have thus much the same distribution as the Balanoglossida, but whereas these latter live in soft black mud the Cephalochorda inhabit clean gravelly sand.

Most of the species are referred to the genus *Amphioxus* (*Branchiostoma*). These animals, as is well known, possess the general form of fish, and their muscles are arranged as in fish, in series of blocks called **myotomes.** They possess a long tubular **spinal cord,** underlaid by an indubitable **notochord** which stretches from end to end of the body, whence the name Cephalochorda. The **pharynx** is pierced by numerous long, narrow gill-slits.

No one has ever questioned the relationship of these animals with the vertebrata. If, therefore, we can discover in their development, features which ally them with the Enteropneusta, the question of the relationship of this latter group with the Vertebrata will be settled in the affirmative.

The mode of development of the eggs of *Amphioxus* was first discovered by Kowalevsky (1867-1877), and was more fully elucidated by Hatschek (1881), whose account has been incorporated in the text-books. The validity of this account was challenged by Lwoff (1894), who has been followed by other workers. We ourselves criticized Lwoff (1898), but our account has in turn been challenged by Cerfontaine (1907), who supports a modification of Lwoff's view, and by Legros (1907). A final answer to Cerfontaine was given by us in 1909. The facts bearing on the controversy will be given in the following pages.

Cerfontaine has given the best account of the segmentation of the egg. He points out that the nucleus of the egg of *Amphioxus* is nearer one pole of the egg than another, and that between this nearer pole and the nucleus no yolk-granules are developed. On this ground he asserts that the egg is not really **alecithal,** but **telolecithal,** and regards this circumstance as a proof that the ancestors of *Amphioxus,* like other Vertebrates, once had large yolky eggs. But this idea is open to serious criticism. In all alecithal eggs, even in those of *Balanoglossus*, the nucleus approaches one pole of the egg when it undergoes division in order to form the first polar body.

The first polar body in the case of *Amphioxus* is formed after the egg is shed from the ovary into the surrounding coelomic space, which is termed the **gonocoele.** From the gonocoele the eggs escape through two slit-like openings into the atrial cavity, along which they pass back to escape by the atrial pore into the sea. In the sea they are fertilized by the spermatozoa which are emitted by the male. Both eggs and sperm are obtained simply by collecting specimens of male and female *Amphioxus* and placing them in jars of clean sea-water. They spawn usually in the evening about 6 P.M. The spermatozoon enters the egg by the pole which is farthest from the nucleus, and only after this happens is the second polar body given off. After this the egg secretes a vitelline membrane, inside which, accordingly, the second polar body is enclosed, and this body remains visible during the earlier part of the development and is used by Cerfontaine as a landmark. The vitelline membrane is formed as in the egg of *Echinus*, by the coalescence of a row of spherical drops emitted from the egg, the outer walls of which coalesce to form a coherent skin. The spermatozoon travels upwards through the egg to meet the egg-nucleus, which descends to meet it. The compound zygote-nucleus is, therefore, nearer the centre of the egg than was the nucleus of the unfertilized egg, *and it is surrounded on all sides by yolk-granules as in any other typically alecithal egg.*

The fertilized egg begins at once to segment, and it is easy to keep the developing eggs in jars of clean sea-water, in which they will live until the larvae hatch out about ten hours after fertilization. These larvae live for a day or more until they have developed a mouth and one gill-slit, further than this stage it has not been found possible to rear them in captivity. Later stages are procured by fishing in the water with a tow-net. The experiment, however, of feeding these larvae on diatoms has never been tried: it is extremely likely that an attempt to do this would be crowned with success.

The eggs, in their earlier stages of development, are best preserved in the mixture of solution of corrosive sublimate and glacial acetic acid; but once the larvae have hatched, indeed once the gastrulation is over, osmic acid is the best preservative. It is necessary to impregnate them thoroughly with this reagent, in order to stiffen their delicate tissues and to prevent their collapsing in the process of dehydration. Also it is absolutely necessary to embed first in celloidin, and then in paraffin, if good results are to be obtained. The minute size of the embryos renders it possible to obtain what is equivalent to a good whole mount, by immersing the fragment of celloidin in cedar oil. The position of the embryo in the celloidin block can, in this way, be accurately ascertained, and so the difficult question of orientation is solved.

The egg divides in the usual way into two and then four blastomeres. Of these four, two, which later are shown to be anterior, are rather smaller than the others, so that even at this early period the egg is bilaterally segmented. These four blastomeres in turn divide into an upper and a lower tier, and so the 8-cell stage is attained; but the four upper cells, termed by Cerfontaine **micromeres**, are rather smaller than the four lower cells termed **macromeres**. There are therefore two larger and two smaller micromeres, and two larger and two smaller macromeres.

In attaining the 16-cell stage each cell divides into right and left daughters by radial planes, so that two tiers of eight cells should be formed; but four macromeres move downward and four micromeres upwards, so that four tiers of four cells each are formed. Each cell then divides into upper and lower halves, and in the 32-cell stage we have actually eight tiers of four cells each. But, as is shown in Fig. 428, this unstable arrangement does not persist. The blastomeres glide on one another, and we get a circle of four cells at both the animal and the vegetative pole, and three intervening tiers of eight cells each. The tier at the animal pole consists of the smallest cells, that at the vegetative pole of the largest, and in the intermediate tiers we have a gradual passage from the one size to the other.

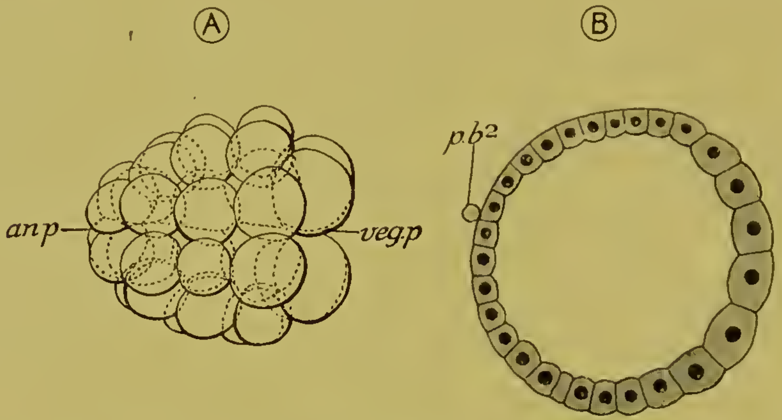

FIG 428.—Stages in the segmentation of the egg of *Amphioxus lanceolatus*. (After Cerfontaine.)

A, 32-cell stage seen from the side. B, optical sagittal section of a young blastula. *an.p*, animal pole of the egg; *pb*^{2}, second polar body; *veg.p*, vegetative pole of the egg.

At the next period of cleavage the planes of division are no longer parallel to one another in all the cells. The equatorial cells divide into right and left daughters, but the planes of division in the polar cells are oblique. After the next cleavage the egg has divided into 128 cells, and these commence to flatten out against each other and to take on the character of a columnar epithelium which is ciliated.

At the next stage, when there are 256 cells, the embryo is a spherical **blastula.** At this stage, if we compare the blastula to a school globe, and the animal pole to the North pole, and if we imagine a traveller passing from the North pole to the South, he would continually encounter larger cells; whilst if he passed round the equator from a point which we may compare to Africa, to a point which we may compare to South America, he would also pass from smaller to larger cells—since the two anterior blastomeres of the 4-cell stage, and all the cells descended from them, are smaller than the two posterior blastomeres and their descendants.

In the next stage the blastula flattens on one side and becomes hemispherical (Fig. 429, A). On the flat side there are the largest cells, which are now of a tall columnar shape. At one edge of the flat surface there is an abrupt passage from cells of this character to comparatively small cells, but at the other edge the tall columnar cells pass gradually into the lower cells which form the hemispherical wall of the blastula. The first edge of the flat surface we may term x, the second y.

The process of **gastrulation** begins within an inflection of the flat surface near the edge named x (Fig. 429, B). The invagination is therefore not a symmetrical invagination of the centre of the lower surface, as in Balanoglossida and Echinodermata, but is such as to give the impression of its being due to the push of an invisible finger directed against the flat surface near the edge x.

This asymmetry in the invagination was not noticed by Kowalevsky and Hatschek, but was first noted by Lwoff, and is made by him the foundation stone of his theory. According to him the invagination occurs in two stages: in the first, the columnar cells, which according to him alone represent the endoderm, are invaginated. Following this stage, however, small cells are inflected at the dorsal lip of the blastopore, and these Lwoff regards as **ectoderm.** These small cells form eventually the roof of the archenteron, and from them notochord and mesoderm are developed, and these structures therefore, on this theory, would be of ectodermal origin. With this view Cerfontaine (1907) substantially agrees, except that he maintains that, at a later stage in gastrulation, ectoderm is invaginated round the ventral lip of the blastopore as well as round the dorsal lip.

Now, it will be observed that Lwoff's case stands or falls with the presumption that, in the hemispherical stage of the blastula, ectoderm and endoderm are already finally separated from one another. If this presumption is ill-founded the whole procedure of splitting the process of gastrulation into two stages is condemned. A careful examination of the hemispherical blastula proves that, except at the edge x, there is no such sharp delimitation of the columnar cells which make up the supposed endoderm, from the supposed ectoderm, as Lwoff postulates. The delimitation at x is seen to be due to the presence there of a zone of rapidly dividing cells, and it is plausible to associate the invagination itself with the pressure exerted by the masses of new cells formed. Indeed, in all cases where the process of gastrulation has been carefully investigated, as for instance in the egg of *Echinus,* invagination is preceded by a rapid multiplication of cells, near the spot on the wall of the blastula which is invaginated.

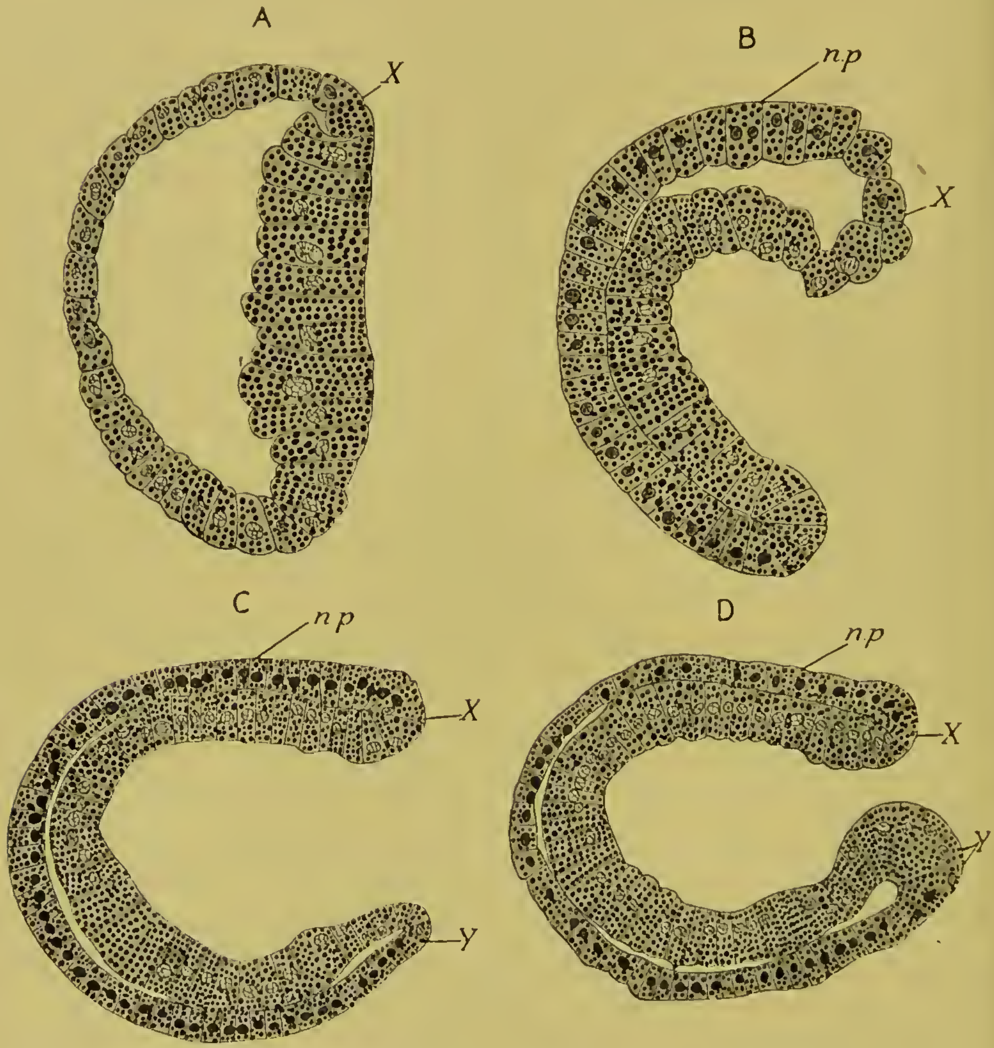

FIG. 429.—Stages in the gastrulation of the egg of *Amphioxus lanceolatus.* All the figures represent median sagittal sections.

A, stage of hat-shaped blastula. B, the invagination of the endoderm well advanced; note the rounded cells at the dorsal lip of the blastopore. C, the gastrula has grown in length—the neural plate is clearly marked. D, the upgrowth of the ventral lip of the blastopore has begun. *n.p*, neural plate; *X*, dorsal lip of the blastopore; *Y*, ventral lip of the blastopore.

There is no ground, however, for assuming an inflection of cells round the dorsal lip of the blastopore, indeed, as invagination proceeds we obtain definite evidence that this is *not* the case. For although at first the nuclei of all the cells of the embryo are alike, being large, clear, and vesicular, with relatively little chromatin, yet soon the nuclei of the cells of the outer layer become more dense and stain with avidity (Fig. 429, B).

This phenomenon appears to be the outward sign of a physiological differentiation between the ectoderm and endodermal cells; and all the invaginated cells, those which were columnar on the flat surface of the hemispherical blastula, and also those which are subsequently invaginated and form the roof of the archenteron, retain clear and vesicular nuclei, and differ from the ectodermal cells in this respect. Further, the rapidly dividing cells at the edge *x*, which, in consequence of the contraction following on completed karyokinesis, are small and rounded, are of two kinds; those which are destined to be added to the ectoderm have deeply staining nuclei, those which are destined to be added to endoderm have vesicular nuclei. Here, then, we have proof that at the edge *x* we have a **meristem** like that of a plant, *i.e.* a growing zone, from the cells produced by which, different layers are differentiated.

The processes which we have been describing lead to the formation of a cup-shaped gastrula with a huge blastopore. As Morgan and Hagen (1900) have shown, there is a general growth in length of the embryo, due to divisions of cells scattered throughout both ectoderm and endoderm. In virtue of this growth the cup becomes more comparable to a thimble.

The rudiment of the first definite organ now appears. This is the **neural plate** (*n.p*, Fig. 429), the so-called "**medullary plate,**" which here, as in all the higher Vertebrata, is the first organ to appear. The ectoderm cells on one surface of the thimble become more columnar than the rest, and this surface becomes slightly flattened, and so the neural plate is formed. The appearance of the neural plate settles definitely the question as to whether edge *x* corresponds to the dorsal edge of the blastopore or not. It also proves that the diameter of the blastopore and the long axis of the nerve plate are at right angles to one another.

The wide blastopore becomes then reduced to a narrow pore by a new process of growth, which starts at the lower lip of the blastopore, in the region corresponding to the lower limit of the flat surface in the hat-shaped blastula, a region which we have already denominated *y*. Here a meristem of dividing cells is formed, exactly like that which, in a previous stage, existed at the edge *x*. By the activity of this second zone an up-growth of the lower ventral lip of the blastopore takes place, and all that is left of the blastopore is a small rounded aperture near the dorsal surface. Fig. 430 is intended to show how much of the endoderm and ectoderm of the gastrula is developed from the walls of the hat-shaped blastula, and how much owes its origin to the two zones of growth, *x* and *y*. The blastopore is closed by the meeting in the middle line of two lateral folds of ectoderm cells termed the **neural folds** (*n.f*, Fig. 431). These flaps originate at the sides of the blastopore and extend forwards along the sides of the neural plate. They meet first over the blastopore and last over the front end of the nerve plate. Here, indeed, for a considerable time an aperture persists—the so-called **neuropore.** It follows that, at the hinder end of the animal, a short vertical canal, covered over by the neural folds, leads upwards from the archenteric cavity to the canal of the nerve chord. This is the so-called **neurenteric canal** (*n.e*, Fig. 431, D).

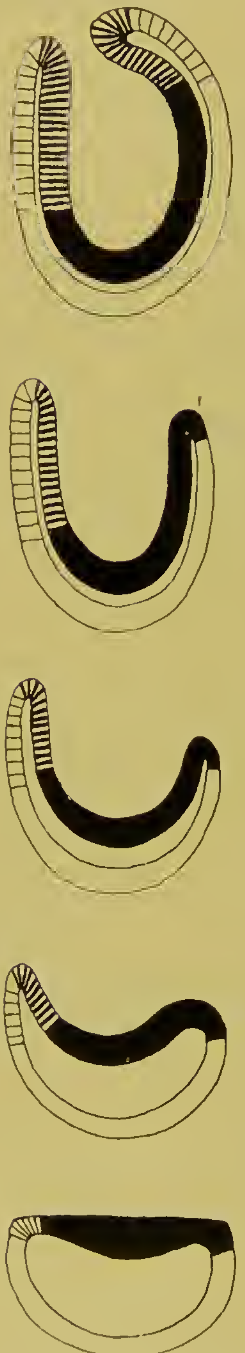

Fig. 430.—Diagrams showing the parts played by ectoderm and endoderm in the process of gastrulation in *Amphioxus lanceolatus.*

Unshaded portion represents the original ectoderm. Deep black portion represents the original endoderm. Portion crossed by thin lines represents the new ectoderm produced by the growing points at the lips of the blastopore. Portion crossed by thick lines represents the new endoderm produced by the growing points at the lips of the blastopore.

In a later stage of development the **anus** opens at the lower end of this canal. The anus may be regarded, consequently, as a remnant of the lower edge of the blastopore, and indeed in the Urodela this portion of the blastopore never closes, but remains permanently open as the anus.

As soon as the blastopore is closed, the formation of the **notochord** and of the coelom takes place. The **notochord** originates from the mid-dorsal region of the archenteron. Here a strip of cells, in which the yolk-granules have disappeared, becomes folded up into an arch (*ch*, Figs. 431, 432), which becomes cut off from the rest of the endoderm as a tube with a minute cavity. This cavity speedily vanishes and the notochord thus becomes a solid rod of cells. The radial arrangement of the cells in the rod soon becomes lost, *i.e.* the cells no longer meet in a point, but interdigitate with one another.

The coelom arises as two pairs of evaginations of the dorso-lateral walls of the gut. The front pair might be described as a pair of pockets, the hinder, more correctly, as a pair of longitudinal **coelomic grooves**, the front ends of which overlap the pockets. The front pairs we shall compare to the **collar-coelomic cavities** (*col.coc*, Fig. 431) of the Enteropneusta, the hinder pair to the **trunk-coelomic cavities** of those animals. The walls of these coelomic sacs constitute the **mesoderm.** After the neural plate is covered over by the meeting of the neural folds, it becomes bent into the form of a U (Fig. 432), and finally, by the meeting of the edges of this, into a tube which is the **neural tube** or **spinal cord.** During the larval life of the animal this tube opens in front by the neuropore (*n.p.o*, Fig. 436, B).

Soon after the formation of mesoderm an extremely rapid growth in length of the embryo sets in, then hatching takes place, and the minute larva swims around by the aid of the cilia which clothe the whole ectoderm. The growth in length is chiefly localized in the hinder region of the embryo, and includes the hinder ends of the trunk-cavities, so that these grow in length *pari passu* with the rest of the larva. There is, however, a general growth in length of all

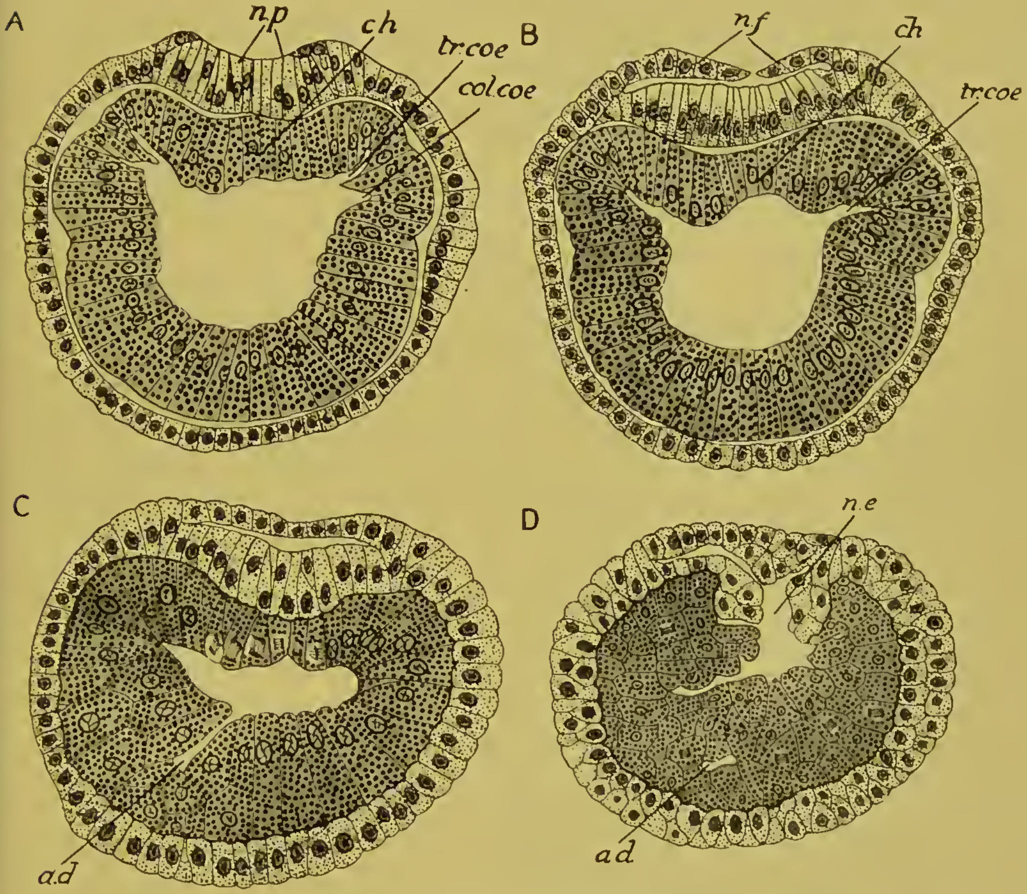

FIG. 431.—Transverse sections through an embryo of *Amphioxus lanceolatus* just after the completion of gastrulation, in order to illustrate the development of nerve cord, notochord, and coelom.

A, section through the anterior region of the embryo, showing the neural plate exposed and the evaginations of the gut, which give rise to collar and trunk coelomic cavities. B, section through a more posterior region of the embryo, showing the neural folds nearly meeting above the neural plate, and the arching of the mid-dorsal section of the gut to form the notochord. C, section through the hinder end of the embryo, showing the anal diverticulum and the union of the neural folds. D, section behind the last, showing the neurenteric canal. *a.d*, anal diverticulum; *ch*, rudiment of the notochord; *col.coe*, rudiment of the collar coelom; *n.e*, neurenteric canal; *n.f*, neural folds; *n.p*, neural plate; *tr.coe*, rudiment of trunk coelom.

parts of the larva, as evinced by the fact that the diameter decreases, up till the end of the second day; and the constituent cells of all the tissues become smaller in transverse section. The left anterior coelomic pocket, or collar-cavity, retains an open connection with the archenteron for a considerable time, and the hinder ends of the trunk-cavities also remain open to the archenteron for a considerable time, whilst their anterior portions are grooved off from it.

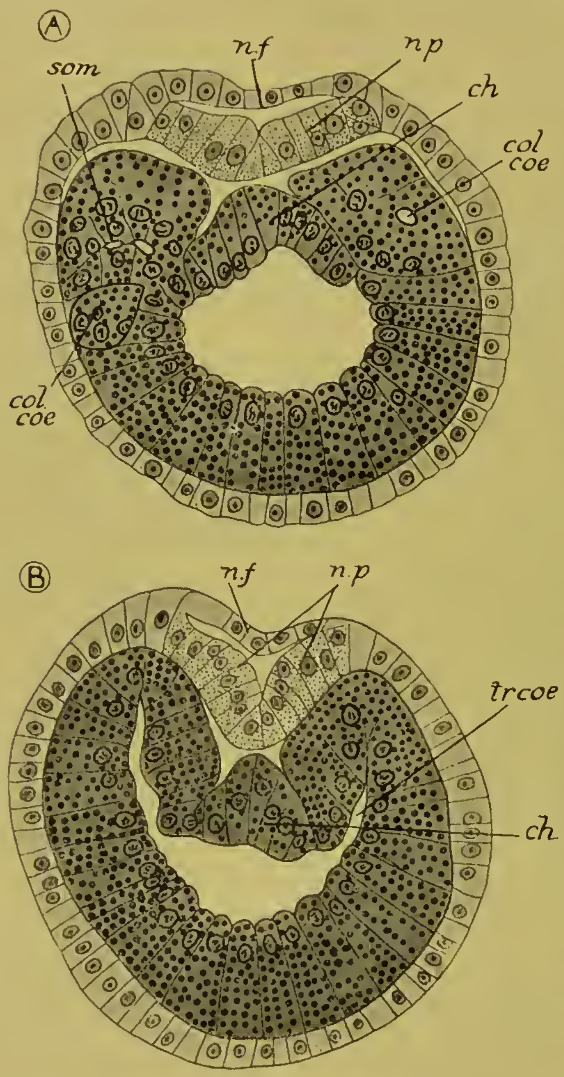

FIG. 432.—Transverse sections through an older embryo of *Amphioxus lanceolatus* than that represented in the previous figure, to show the further development of nerve cord, notochord, and coelom.

A, anterior section, showing on the left the first somite cut off from the front end of the trunk coelom, on the right the collar-cavity. B, posterior section, showing the open groove in which the trunk coelom terminates posteriorly. *ch*, rudiment of notochord; *col.coe*, collar coelom; *n.f*, neural fold; *n.p*, neural plate; *som*, somite; *tr.coe*, trunk coelom.

These anterior parts become divided into segments which are called **somites** (*som*, Fig. 432). The first pair of somites are formed from the collar-cavities. The somites are at first small spherical vesicles situated at the dorso-lateral angles of the gut. They have at first only minute cavities. They soon begin to grow downward between gut and ectoderm, and the cells forming their walls become, for the most part, thin and flattened. An exception to this statement is constituted by the cells forming the inner walls of the somites, where these walls abut on the notochord. These cells remain columnar in cross-section, and develop fibrils in their cytoplasm, and so form the **longitudinal muscles** of the larva.

When five or six somites have been formed, that portion of the archenteron which lies in front of the anterior pockets, or collar-cavities, becomes grooved off from the rest, and simultaneously divided into right and left cavities, of which the right is larger than the left. These are the so-called **head-cavities**, which we have compared to the proboscis-cavity of Enteropneusta. The remainder of the archenteron now constitutes the **gut.**

Meanwhile the somites derived from the trunk-cavities become divided into upper portions, termed **myotomes,** from whose inner walls the muscles are formed, and lower portions, which fuse with their predecessors and successors to form the general body-cavity or **splanchnocoele.** The right and left splanchnocoeles meet with one another and fuse into one cavity in the mid-ventral line beneath the gut. Above the gut they remain separate from one another, and between them lies a blastocoelc space, double in front but single behind, which is the rudiment of the **dorsal aorta.**

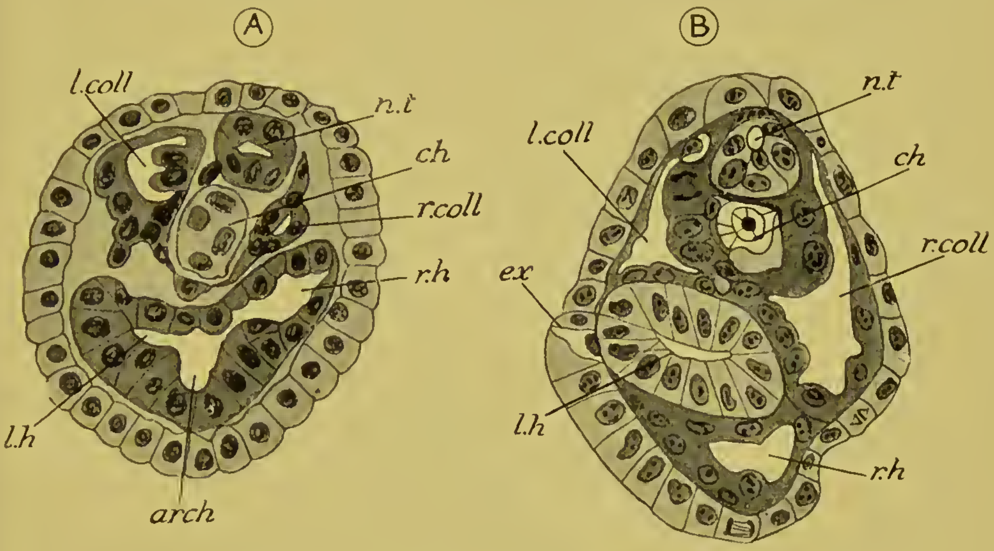

Fig. 433.—Illustrating the formation and development of the head-cavities of *Amphioxus lanceolatus.*

A, transverse section through the anterior region of a larva with six myotomes—the head-cavities are seen to be developing as outgrowths of the front end of the gut (archenteron). B, transverse section through the anterior region of a larva two days old just before the mouth opens—the left head-cavity is a thick-walled vesicle; the right head-cavity is a thin-walled vesicle and lies beneath the left one. *arch*, archenteron (gut); *ch*, notochord; *ex*, vacuolation of ectoderm where the external opening of the left head-cavity will be formed later; *l.coll*, left collar-cavity; *l.h*, left head-cavity; *n.t*, nerve tube; *r.coll*, right collar-cavity; *r.h*, right head-cavity.

Since new somites are being continually cut off from the hinder parts of the trunk-cavities, all stages in the development of a somite may be made out in one and the same individual. The somites derived from the collar-cavities, however, do not become subdivided like the rest, nor do they fuse with the splanchnocoeles. In early larval life, at any rate, these lower parts constitute cavities lying at the ventro-lateral regions of the animal, outside the splanchnocoeles, whilst the upper portions of the collar-cavities, above the myotome region, extend forwards at the sides of the nerve cord. The general form of the collar-cavities, as seen from the side, is therefore that of a reversed S.

The next changes which occur are, the enlargement of the front end of the gut as the rudiment of a **pharynx**, and the formation of the **mouth**. The mouth appears on the left side of the animal; the pharyngeal wall adheres at this spot to the ectoderm and a perforation takes place (*m*, Fig. 435). Soon after this the left head-cavity, which is small and spherical and lined with columnar cells, fuses with the ectoderm and acquires an opening to the exterior; whilst the right one becomes thin-walled, and extends to the extreme anterior end of the larva beneath the notochord (Fig. 433, B). It is known henceforth as **Hatschek's pit**.

The first **gill-slit** is formed in the mid-ventral line by the union of a downgrowth of the pharynx with an ectodermal ingrowth. Simultaneously a structure called the **club-shaped gland** makes its appearance. This arises as a pocket, high up on the right wall of the pharynx opposite the mouth. This pocket curves downward and forward, and opens to the exterior below and in front of the mouth. Below the opening of the club-shaped gland the wall of the pharynx becomes modified to form a V-shaped ridge, with the apex of the V pointing inwards. The ridge consists of glandular cells and of cells carrying short cilia, and it is the rudiment of the **endostyle** of the adult (*endst*, Figs. 436, 437, 439, 441).

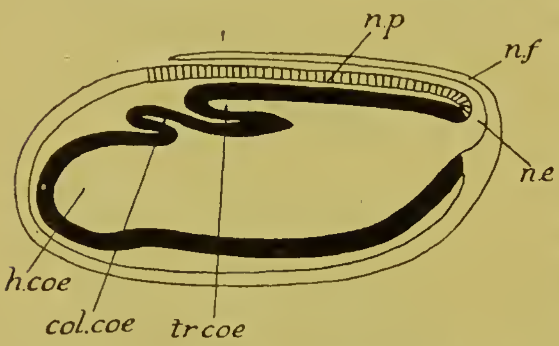

FIG. 434.—Diagram of longitudinal sagittal section of *Amphioxus lanceolatus* to one side of the middle line, in order to show the mutual relationships of head, collar, and trunk cavities. *col.coe*, collar coelomic pouch; *h.coe*, head coelomic pouch; *n.e*, neurenteric canal; *n.f*, neural fold; *n.p*, neural plate; *tr.coe*, trunk coelomic pouch.

This extraordinary larva, which now possesses mouth and anus, and is able to feed itself, leads an active swimming life for about three months. During this period new somites are added behind, and new gill-slits appear in a single series behind the first; but they are no longer mid-ventral, but are actually situated on the right side of the larva. The development of these has been worked out by Lankester and Willey (1890), who term them **primary gill-slits**. As many as fourteen (according to Goodrich [1910] fifteen) of these primary gill-slits are formed.

The hinder ends of the coelomic grooves or trunk-cavities have by this time become solid and have finally separated from the gut: the neurenteric canal also has become a solid string of cells, and this, and the neighbouring tissues, give rise to a growing zone, the **tail-bud**. From this bud there grows out a post-anal extension of nerve cord, notochord, and myotomes, known as the **tail** (Fig. 437).

The tail is a most characteristic organ of the Vertebrata. It is true that the name "tail" is often loosely applied to the posterior part of the body of any animal, if it happens to be thin and flexible, but its proper application is to a post-anal extension of the body

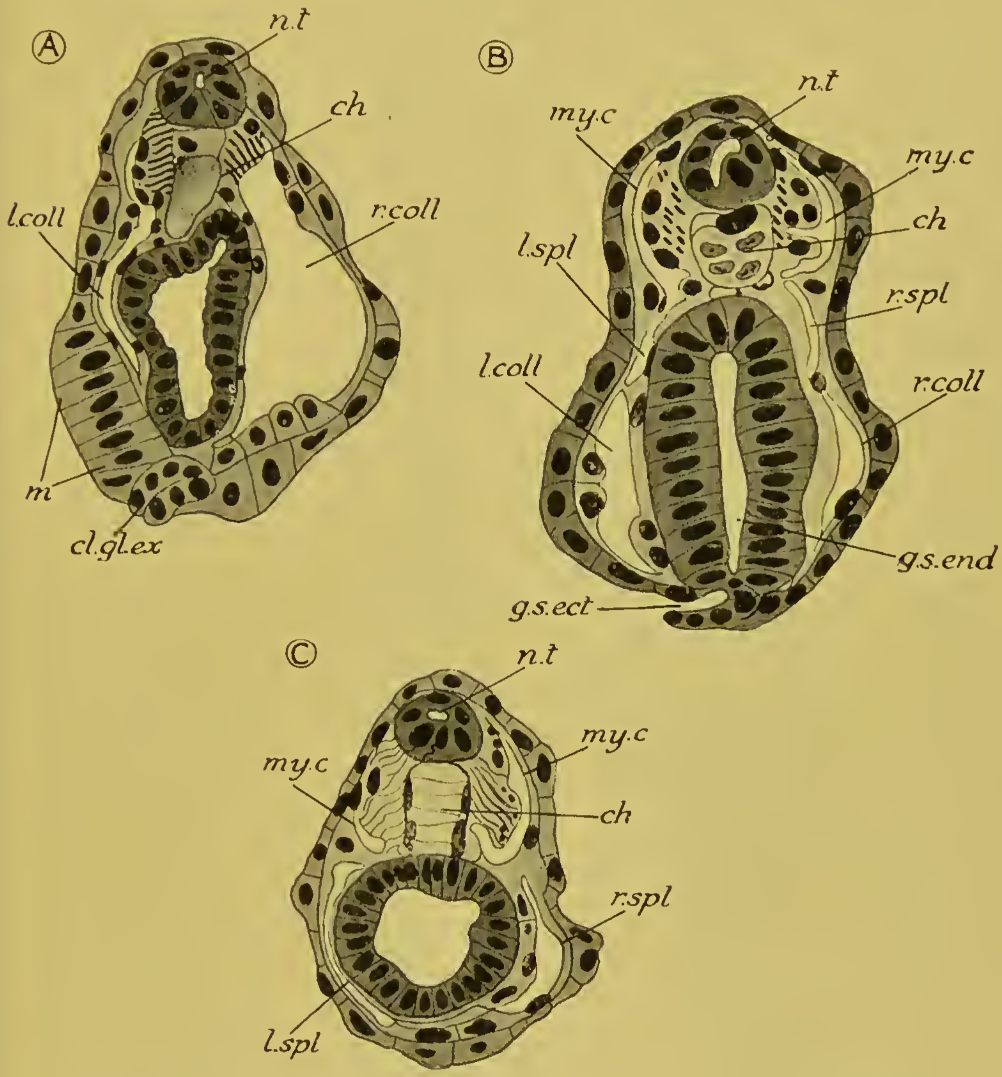

FIG. 435.—Illustrating the mutual relationships of collar-cavities and splanchnocoeles in *Amphioxus lanceolatus*.

A, transverse section passing just in front of the mouth of a larva, in which the mouth and first gill-slit have been formed—the collar-cavities lie at the sides of the pharynx. B, transverse section through the region of the first gill-slit of a larva in which the mouth has not yet been formed—the splanchnocoeles appear pressing the collar-cavities downwards towards the ventro-lateral angles of the pharynx; the gill-slit is seen to be formed by the meeting of ectodermal and endodermal outgrowths. C, transverse section through the hinder end of the pharynx of a larva in which the mouth and first gill-slit have been formed; the collar-cavities have disappeared. *ch*, notochord; *cl.gl.ex*, external opening of the club-shaped gland; *g.s.ect*, *g.s.end*, ectodermal and endodermal rudiments of gill-slit respectively; *l.coll*, left collar-cavity; *l.spl*, left splanchnocoele; *m*, front lip of mouth; *my.c*, myocoele; *n.t*, nerve tube; *r.coll*, right collar-cavity; *r.spl*, right splanchnocoele.

formed as a secondary outgrowth from a posterior zone of growth.

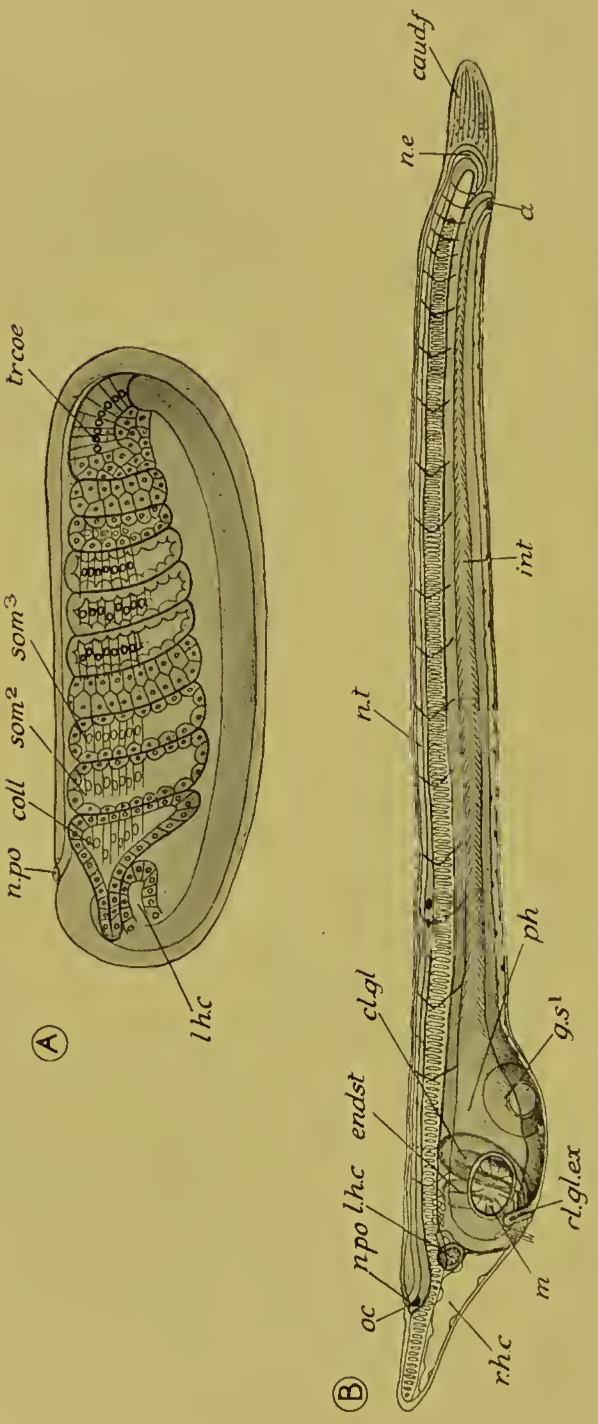

Fig. 436.—Two young larvae of *Amphioxus lanceolatus* seen from the left side. (After Hatschek, slightly altered.)

A, stage of formation of head-cavities, eight somites and the collar-cavities formed. B, stage after opening of mouth and first gill-slit. *a*, anus; *caud.f*, caudal fin; *cl.gl*, club-shaped gland; *cl.gl.ex*, external opening of the club-shaped gland; *coll*, collar-cavity; *endst*, endostyle; *g.s*¹, first primary gill-slit; *int*, intestine; *l.h.c*, left head-cavity; *m*, mouth; *n.e*, neurenteric canal; *n.po*, neuropore; *n.t*, nerve tube; *oc*, eye-spot; *ph*, pharynx; *r.h.c*, right head-cavity; *som*², *som*³, the second and third somites (the first somite is formed by the collar-cavity); *tr.coe*, trunk coelom.

In many fish the tail is several times longer than the body, and it is characteristic of the low place which *Amphioxus* occupies in the vertebrate phylum as compared with fish that its tail-growth is insignificant.

In older larvae a slight ectodermic thickening along the line of the posterior extension of the first somite, on the right side, is observable; this thickening is the first rudiment of the **atrial fold,** which overhangs the gill-slits. A similar fold is developed on the left side at a later period of development, and during the metamorphosis into the adult form these two folds unite beneath the ventral surface of the animal and enclose the **atrial cavity.**

Hollow outgrowths from the inner walls of the myotomes give rise to tubes which insinuate themselves between the myotomes on the one hand and notochord and nerve cord on the other (Hatschek, 1888). These are the **lower sclerotomes** (*scl*², Fig. 438); from their inner walls a fibrous sheath is developed which enswathes notochord and nerve cord. Their outer walls furnish a sheath, or **fascia,** for the muscular fibres of the myotomes.

From the uppermost angles of the myotomes similar outgrowths arise which we may term the **upper sclerotomes** (*scl*¹, Fig. 438), and become completely cut off from the myotomes. Thus they give rise to a series of pockets lined by coelomic cells which are termed the **fin-ray cavities.** These cavities were described by Hatschek (1888); their origin from the myocoele was first seen by Goldschmidt (1905). The cavities are arranged in a single series in the mid-dorsal line, four or five occupying a space corresponding to the breadth of a myotome. They appear to be formed by outgrowths arising alternately from the myotomes on the right and left side. The ventral wall of each becomes thickened and forms the gelatinous peg known as the **fin-ray.**

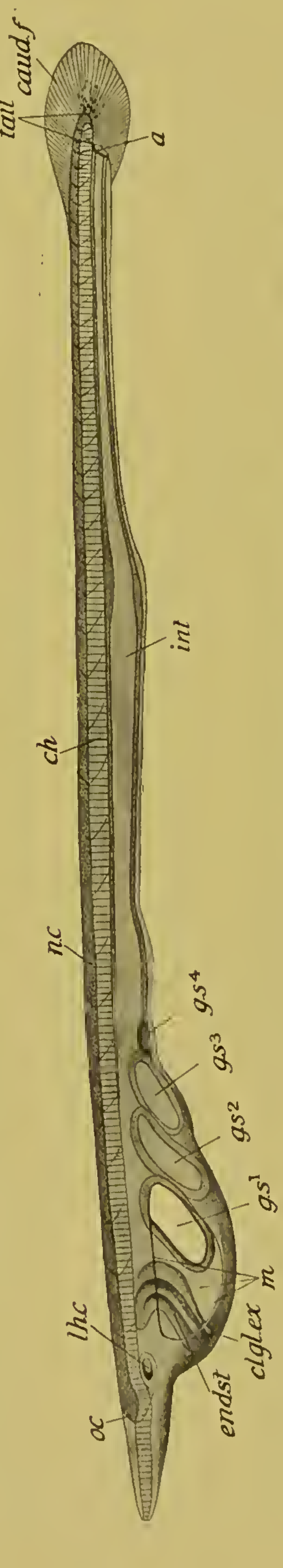

FIG. 437.—Larva of *Amphioxus lanceolatus* with four primary gill-slits seen from the left side. (After Lankester and Willey.)

a, anus; *caud.f*, caudal fin; *ch*, notochord; *cl.gl.ex*, external opening of the club-shaped gland; *endst*, endostyle; *g.s*¹-*g.s*⁴, the four primary gill-slits; *l.h.c.* left head-cavity; *m*, mouth; *n.c* nerve cord; *oc*, eye-spot.

During the larval life the **excretory organs** are formed. These are true **nephridia** comparable to those of Oligochaeta, Nemertinea, and Platyhelminthes. They consist of tubes ending internally in tufts of **solenocytes** or flame cells. Their origin has been described by Goodrich (1909). When they are first discernible they consist of small pear-shaped masses of cells, almost certainly ectodermal in origin, situated at the lower borders of the primitive gill-slits. They rapidly become hollow and long solenocytes grow out from their inner ends. These solenocytes extend up the left side of the body to the level of the dorsal aorta (*sol*, Fig. 439). In the adult the solenocytes are much shorter than in the larva. One very large nephridium lies in the forward extension of the right collar-cavity, and opens behind into the mouth. This is called **Hatschek's nephridium.**

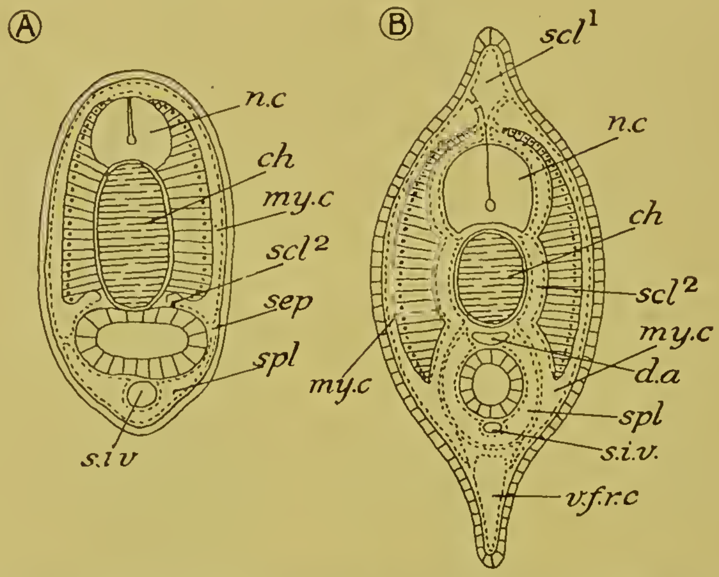

FIG. 438.—Diagrammatic transverse sections through the hinder region of two young specimens of *Amphioxus lanceolatus*, in order to show the origin of the sclerotomes. (After Hatschek, slightly altered.)

A, through a larva with five primary gill-slits—the lower sclerotome is just beginning to be formed. B, through a specimen just after the metamorphosis—the lower sclerotome has extended upwards between the myotome and the notochord, and the upper sclerotome has given rise to the fin-ray cavity. *ch*, notochord; *d.a*, dorsal aorta; *my.c*, myocoele; *n.c*, nerve cord; *scl*^{1}, upper sclerotome; *scl*^{2}, lower sclerotome; *sep*, septum dividing myocoele from splanchnocoele; *s.i.v*, sub-intestinal vein; *spl*, splanchnocoele; *v.f.r.c*, ventral fin-ray cavity.

As the larva grows older it seeks lower and lower levels in the water. Finally, after about three months of larval life, the metamorphosis sets in, and during this period the larva frequently lies on its side on the bottom. Before metamorphosis begins, however, the rudiments of the **genital organs** make their appearance. Their development has been elucidated by Boveri (1892). They arise as small buds of cells (*germ*, Fig. 440), which sprout from the anterior lower angles of the myotomes, and which project into spaces derived from the posterior lower angles of the **myocoeles,** as the cavities of the **myotomes** are

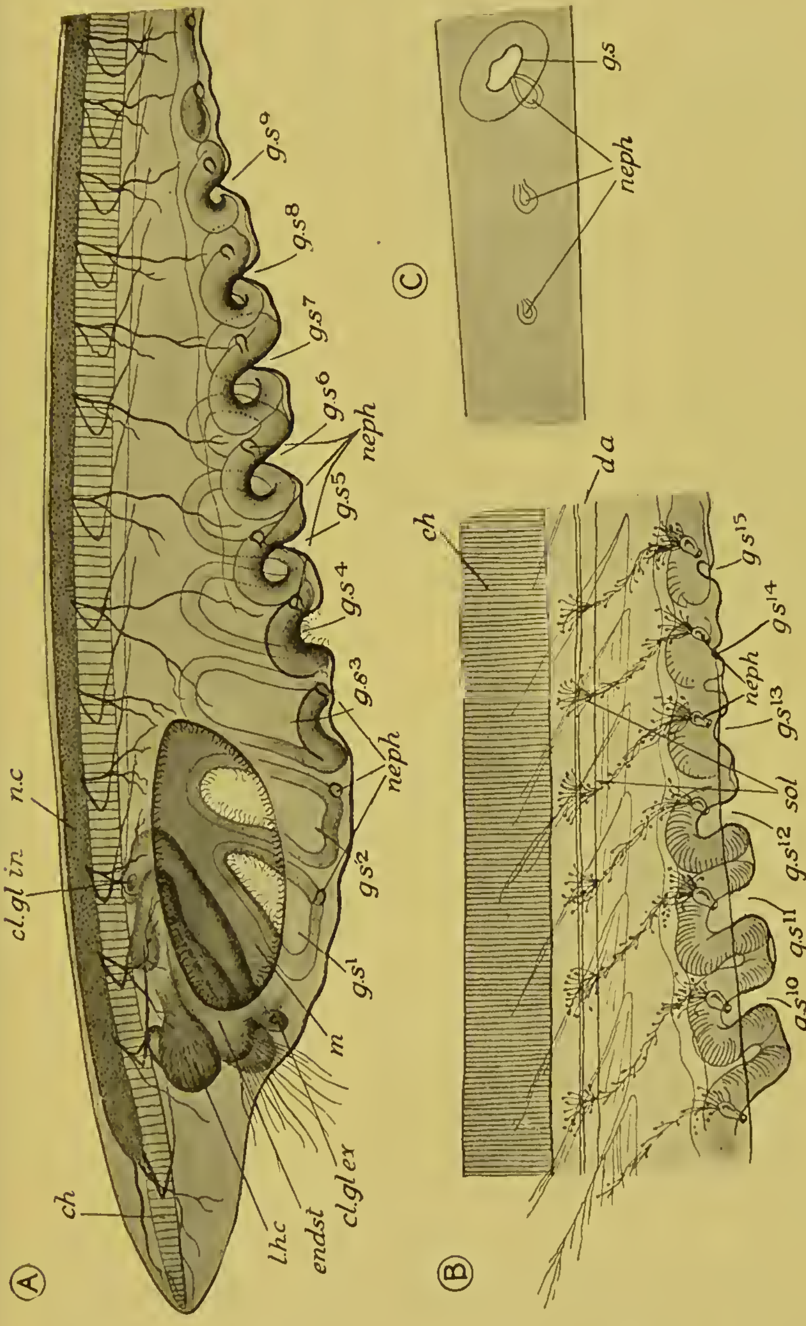

FIG. 439.—Illustrating the development of the excretory organs of *Amphioxus lanceolatus.* (After Goodrich.)

A, left side-view of a larva, showing the earliest rudiments of the nephridia at the lower borders of the primary gill-slits. B, posterior branchial region of a larva, more highly magnified, seen from the left side, showing the very long larval solenocytes of the nephridia. C, the end of the branchial region of a larva seen from the ventral surface, showing the last-formed gill-slit and two nephridia posterior to it (the anterior end of this figure is to the right). *ch*, notochord; *cl.gl.ex*, external opening of the club-shaped gland; *cl.gl.in*, internal opening of the club-shaped gland; *endst*, endostyle; *g.s*$^{1\text{-}15}$, the fifteen primary gill-slits; *l.h.c*, left head-cavity; *m*, mouth; *n.c*, nerve cord; *neph*, rudiment of nephridium; *sol*, larval solenocyte.

called. These spaces form the **gonocoeles** of the adult; they soon become cut off from the myocoeles from which they are derived by the growth of septa, and each gonad projects into the gonocoele derived from the myocoele in front of the one to which it belongs.

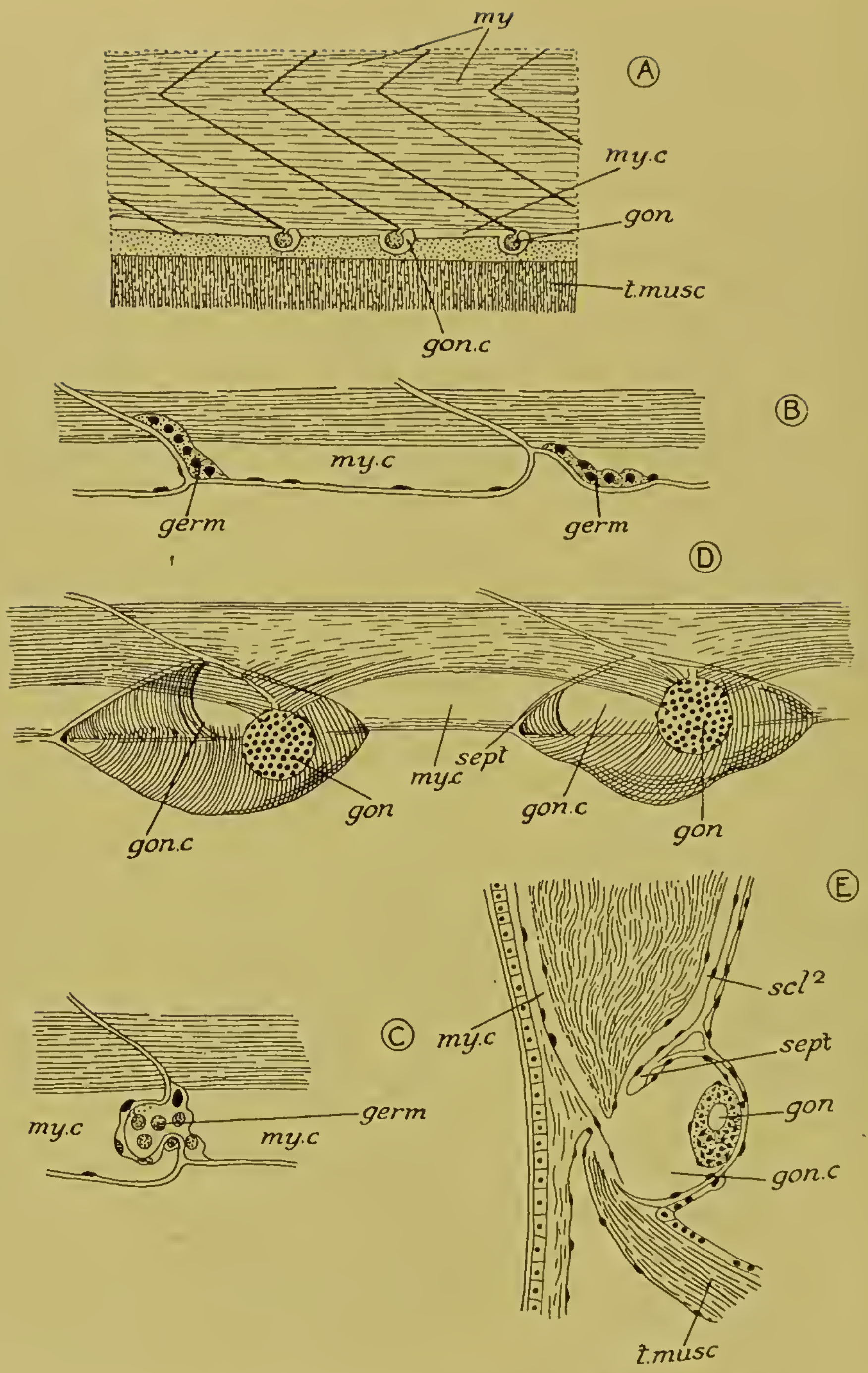

FIG. 440.—Illustrating the development of the genital organs of *Amphioxus lanceolatus*. (After Boveri.)

A, a portion of the body-wall of a young *Amphioxus*, 9 millimeters long, viewed as a transparent object, in order to show the relation of the genital rudiment to the myotomes. B, a small portion of a similar preparation from a still younger specimen, in order to show the origin of the germ cells. C, a small portion of a similar preparation from a slightly older specimen than that from which B is taken, to show the indentation of the wall of the myotome by the genital rudiment. D, a small portion of a similar preparation from a specimen 14 millimeters long, to show the formation of the gonocoele from the myocoele. E, a portion of a transverse section through a specimen of the same size as that from which D is taken. *germ*, primitive germ cells; *gon*, rudiment of genital organ; *gon.c*, gonocoele; *my*, myotome; *my.c*, myocoele; *scl²*, lower sclerotome; *sept*, septum, dividing gonocoele from myocoele; *t.musc*, transverse muscle in the floor of the atrial cavity.

The changes which occur during metamorphosis have been described in detail by Willey (1891). The first gill-slit and the club-shaped gland disappear simultaneously. Several of the more posterior of the "primary" gill-slits also disappear, and in this way

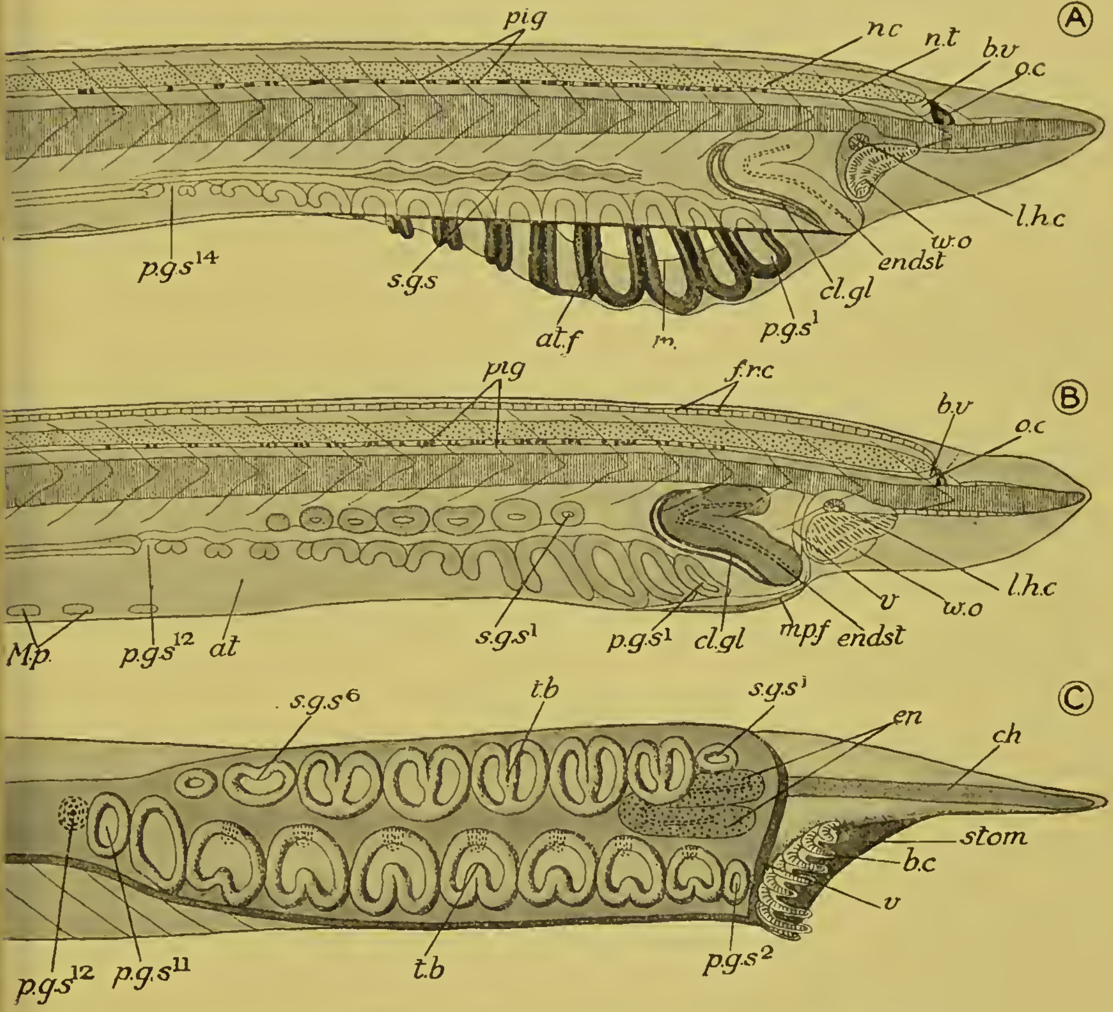

FIG. 441.—Illustrating the metamorphosis of the larva of *Amphioxus lanceolatus*. (After Willey.)

A, larva, with full number of primary gill-slits, seen from the right side; six thickenings of the pharyngeal wall above the primary gill-slits indicate the beginnings of the secondary gill-slits; the atrial folds are not yet united. B, larva in which the hindermost primary gill-slits have disappeared; six secondary gill-slits have been formed and the atrial folds are united; viewed from the right side. C, larva in which eight secondary gill-slits have been formed, five of which are divided by tongue-bars; the primary gill-slits have been reduced to nine, and tongue-bars have begun to be formed in them; the endostyle has been shifted to the mid-ventral line, and oral cirri are beginning to be formed; viewed from the ventral side. *at*, atrial cavity; *at.f*, atrial fold; *b.c*, buccal cavity; *b.v*, brain-vesicle; *ch*, notochord; *cl.gl*, club-shaped gland; *en*, *endst*, endostyle; *f.r.c*, fin-ray cavities; *l.h.c*, left head-cavity; *m*, border of mouth; *M.p*, Müller's papillae; *m.p.f*, metapleural fold; *n.c*, nerve cord; *n.t*, neural tube; *oc*, eye-spot; *p.g.s*¹, *p.g.s*², *p.g.s*¹¹, *p.g.s*¹², *p.g.s*¹⁴, the first, second, eleventh, twelfth, and fourteenth primary gill-slits; *pig*, pigment spots in nerve cord; *s.g.s*¹, *s.g.s*⁶, the first and sixth secondary gill-slits; *stom*, stomodaeum; *t.b*, tongue-bar; *v*, velum; *w.o*, wheel-organ.

the number of these is reduced to eight. A new series of eight gill-slits make their appearance on the right side of the larva, above the primitive gill-slits. These new gill-slits (termed by Willey the **secondary gill-slits**) grow downwards, pressing the primitive gill-slits back to the mid-ventral line of the larva, and eventually on to its left side. Thus the "primary" gill-slits form the adult gill-slits of the left side, and the "secondary" gill-slits those of the right side. In this way the lower borders of the primary gill-slits become the dorsal borders, and the excretory organs reach the position in which they are found in the adult, viz. the dorsal border of each gill-slit.

At the same time, in both primary and secondary gill-slits, septa grow out from their dorsal edges, extend across them, and eventually divide each of them into two. These septa are the **tongue-bars** (*t.b*, Fig. 441). The endostyle, which like the gill-slits lay originally on the right side of the pharynx, is forced downwards to the mid-ventral line; it grows backwards until it extends along the whole length of the pharynx. The **buccal cavity** or **stomodaeum** is formed by the outgrowth of two cheek-like folds from the collar region (region of the first myotome), which extend forwards beneath the notochord, and enclose between them a space which formerly was part of the external world, lying in front of the larval mouth. The larval mouth persists at the bottom of the stomodaeum as an opening in the so-called **velum**, leading into the pharynx.

On the dorsal surface of the buccal cavity an extensive series of ciliated grooves makes its appearance. These grooves radiate from the opening of the left head-cavity, now termed Hatschek's pit, and their function seems to be to carry off the secretion of this pit. They are termed collectively the **wheel-organ** (*w.o*, Fig. 441). At the side of the buccal cavity the ciliated rods, known as **oral cirri**, grow out (Fig. 441, C). The atrial ridges unite to form the floor of the atrial cavity; on their inner surfaces ectodermic thickenings appear which are believed to be excretory in function, and are termed **Müller's papillae** (Fig. 441, B).

The metamorphosis may now be regarded as complete, and the animal takes up its habitat in a vertical burrow in the sand. In this burrow it leads a sedentary life, only occasionally emerging from it to make a rapid dash through the water and then seek a new burrow.

The young *Amphioxus* has still to produce new gill-slits, since it has only eight pairs to start with; and it has only the rudiment of a liver. As the animal increases in size, new gill-slits gradually appear behind the existing gill-slits on each side, and a full-grown specimen may have as many as 100 pairs. Each gill-slit begins as a simple perforation which is then bisected by the growth of a tongue-bar. The **liver** begins as a little ventral pouch of the gut immediately behind the pharynx. This pouch grows forward and becomes pushed up on to the right side of the animal. Eventually it becomes so long that its tip reaches nearly to the velum.

THE AFFINITIES OF AMPHIOXUS WITH THE ENTEROPNEUSTA

We have now to endeavour to answer two questions, viz. (*a*) Does the developmental history of *Amphioxus*, as we have sketched it, afford support to the idea that the Enteropneusta have vertebrate affinities; and (*b*) How are we to explain the extraordinary asymmetrical larva, which nevertheless becomes metamorphosed into an adult which is nearly bilaterally symmetrical?

With regard to the first question, it must be remembered that even if Enteropneusta be admitted to the Vertebrate phylum, they must, nevertheless, be widely separated from Cephalochorda. Between a grade of structure in which the neural tube was confined to the collar region, and in which the notochord is represented by a small anterior diverticulum of the pharynx destined to support the base of the proboscis region; and a grade of structure in which neural tube and notochord extend throughout the entire length of the body, an enormous gap intervenes, to span which an enormous lapse of time must have been required.

Nevertheless, in the segmentation of the egg, and in the formation of the layers, there is a fundamental agreement between Enteropneusta and Cephalochorda. The segmentation of the egg of *Dolichoglossus*, as sketched by Davis, appears to be of the same general type as that of the egg of *Amphioxus*. The same hemispherical blastula stage is found in *Amphioxus* and in *Balanoglossus*, as described by Heider; and in both cases the archenteron is large and nearly fills the blastocoele, and the embryo, immediately after gastrulation, grows markedly in length. In both cases too (if we follow Morgan [1891] in his account of the development of the New England Tornaria larva), the mesoderm arises from the archenteric wall as five outgrowths, viz. an anterior unpaired pouch and two pairs of lateral pouches. In both the neural plate arises in the same way, as do also the protecting flaps of ectoderm which cover it in. In both it is eventually converted into a neural tube. In both the left division of the anterior coelomic pouch opens to the exterior by a pore. When we add to these resemblances the anatomical similarities between the adult forms in respect of notochord, gill-slits, and tongue-bars, it will be admitted that a very strong case for the vertebrate affinities of the Enteropneusta has been made out. We have not to reconstruct a single organ, and we take as our starting-point a very simple free-swimming animal represented by the Tornaria larva.

On any other theory of the origin of Vertebrata, such as the idea that they came from Annelida, a new mouth has to be manufactured and other radical changes in function have to be postulated, for which there is no precedent in the evolutionary changes which we can determine with moderate certainty.

From the conclusion that the Enteropneusta are Vertebrata, some very interesting consequences follow. The proboscis-cavity of the Enteropneusta is represented by the "head-cavities" of *Amphioxus*, as we have seen, and by the **premandibular cavities** of higher vertebrates, from the walls of which most of the eye muscles arise. Now in the Tornaria larva we find at the apex of the proboscis an apical plate of nervous and sensory cells on which two eye-cups are situated; and in the embryos of the frog the optic areas can be recognized as pigmented spots in the region of the fore-brain whilst this is still an open plate.

It appears probable, therefore, that the ectoderm covering the proboscis of the Enteropneusta corresponds to the fore-brain of Vertebrata, whilst the collar region of the Enteropneusta corresponds to the region of the mid-brain of Vertebrata and what lies beneath it, viz. the **mandibular cavities,** from the walls of which jaw muscles and the superior oblique muscles of the eyes arise. The mandibular cavity would then correspond to the collar-cavity of Enteropneusta and to the region of the first myotome in *Amphioxus.* How far the ventral extension of the first myotome, which is so clear and distinct from the splanchnocoele in the early larva of *Amphioxus,* corresponds to the system of cavities in the lips of the adult, called by Van Wijhe (1901) collectively the **stomocoele,** is a matter requiring further investigation.

The connective tissues and peritoneal membranes of the larvae of *Amphioxus* are difficult to preserve and stain, only mixtures containing osmic acid seem to confer on them the power of taking up stains so as to be clearly distinguishable. The slit-like coelomic cavities are apt to collapse, and then it is impossible to be sure of their extension and limits, as their adherent walls look like single membranes. The best results are obtained by staining the sections, after preliminary stain in bulk with haematoxylin or eosin, with a 1 per cent aqueous solution of picronigrosin.

But the working out of the larval development under conditions like these is still a task for the future. If we assume that the lip-cavities of the adult *Amphioxus* arise from portions of the collar-cavities of the embryo, and that the collar-cavities are homologous structures in *Amphioxus* and Enteropneusta, then we may note that the ciliated **oral cirri,** which aid *Amphioxus* in obtaining its food, are outgrowths of the lip-cavities. These cirri may then, perhaps, be compared to the ciliated tentacles which grow out from the collar-cavities in Cephalodiscida, and to the radial canals, and the tube feet, which are branches of these canals, in Echinodermata. These canals and their branches are outgrowths of the left hydrocoele, which we may compare to the left collar-cavity of *Amphioxus* and Enteropneusta.

When we endeavour to answer the second of our two questions we must admit that the larvae of *Amphioxus* are so extraordinary as to present a serious problem for those who, like ourselves, believe that the larval stage represents, in however modified form, an ancestral condition of affairs. Balfour considered them so abnormal that, when they were first described by Kowalevsky, he thought they must be pathological. That a bilaterally symmetrical embryo should pass through an asymmetrical pelagic larval stage, in order to attain the form of a practically symmetrical burrowing adult, seems to be in complete contrariety to what we should expect.

But a very plausible solution has been suggested by Stafford (quoted in MacBride, 1909). He points out that the ancestor of *Amphioxus* could not have suddenly jumped from a pelagic to a burrowing existence. Some intermediate condition of life must have existed. What was the intermediate state of affairs? In the case of other animals which have deserted a pelagic for a more humble form of existence, we have reason to believe that a habit of gliding over the surface of the mud preceded a burrowing life. But a vertically flattened animal like *Amphioxus* could not do this and keep on an even keel. The moment it ceased swimming it must have tended to fall on one side. This circumstance can actually be observed in the older larvae of *Amphioxus.* Let us assume, therefore, that the ancestors of *Amphioxus,* after deserting their free-swimming life, passed through a stage when they lay on their left sides on the bottom. Under these circumstances it would be an advantage to twist the mouth round to the left side, so as to bring it near the detritus which served as food, and to twist both sets of gill-slits round to the upper right side, so that the waste water might be ejected without disturbing the substratum on which the animal was feeding. Similar conditions of life have led the modern flat-fish to become asymmetrical, they have twisted the mouth down and the two eyes round to the upper side of the body.

But if this solution sounds plausible two further questions may be asked, viz. (*a*) Why does only one row of gill-slits develop? and (*b*) Why does the asymmetry appear at that stage of its development when, to judge from its environment, the larva ought to be bilaterally symmetrical?

The answer to the first question has an important bearing on the *way* in which ancestral adult organs are represented in the larva. The larva in almost every case (see Chap. I.) has been much reduced in *size* in comparison with the ancestor which it represents. If larval organs were reduced on the *same scale* as the whole animal they might be reduced to dimensions which would render their functions difficult, if not impossible of performance. Too narrow gill-slits, for instance, might become useless owing to the viscosity of water in passing through small apertures. Hence arises a tendency, which we have noted elsewhere, to sacrifice *number* to size; thus, of the two series of gill-slits which the ancestor undoubtedly possessed, only one is developed in the larva in order that the individual slits may have room to spread. The missing gill-slits are held over, so to speak, till adult habits are about to be taken up.

That a left-sided mouth and asymmetrical gill-slits should appear in a free-swimming larva is only another example of an exceedingly widespread phenomenon, viz. the reflection of features belonging to later stages of development into earlier stages of ontogeny. This phenomenon, termed **heterochrony** by Lankester, which meets the comparative embryologist at every step of the road on which he travels, is one for which no adequate explanation has as yet been suggested, but it seems to us that it is of the utmost interest in weighing theories of heredity and of the origin of variation.

In conclusion, therefore, we maintain that the asymmetries of the larva of *Amphioxus* are due to the existence of a former "flat-fish" condition, but that these asymmetries have been reflected backwards into a previous free-swimming condition so as to appear in the pelagic larva.

We have now placed before the reader the arguments, which seem to us convincing, that the Enteropneusta are related to the vertebrate phylum. As we have already stated, however, all zoologists are not in accord with this view. We have not here discussed the objections which have been raised to it, because, in the second volume of this work, the reader will have an opportunity of learning at first hand the arguments on the other side, and will then be best able to weigh both sides of the case for himself.

UROCHORDA

The group of Urochorda, more familiarly known as Tunicata, comprises animals, the vast majority of which are sessile, and many of which have much the same external appearance as sponges. By the older zoologists they were classed, with Polyzoa, as Molluscoida. It never entered into the imagination of any one that they had affinities with the Vertebrata till Kowalevsky (1867) showed that the larva possessed a notochord, a tubular dorsal nerve cord expanding in front into a brain-vesicle, and a pair of gill-slits. The larva has a long post-anal **tail** to which the notochord is confined, and on this account the name Urochorda (οὐρά, a tail) is given to the group. Since a brain-vesicle has been developed at the anterior end of the spinal cord, and since there is a tail, we are driven to conclude that the Urochorda must have branched off from the Vertebrate stem when the Vertebrate stock had evolved far beyond the level represented by the Cephalochorda; for in this latter group there is a mere rudiment of a brain-vesicle, and the tail is only beginning.

The Urochorda include a few minute forms termed **Larvacea**, which retain tail, notochord, neural tube, and brain-vesicle throughout life, and remain free-swimming. About the development of these animals little or nothing is known. There is also a small group termed the **Thaliacea** which has secondarily acquired free-swimming habits; the larval tail and notochord are lost in the adult, but the sphincter muscles, which in ordinary forms surround mouth and anus, are developed into hoop-like bands, and by the peristaltic action of these muscles progression is effected.

The vast majority of Urochorda, termed the **Ascidiacea**, are sessile forms which increase by budding. In one small sub-group, the **Ascidiae luciae,** the colony has become free-swimming; in another sub-group, the **Ascidiae simplices,** the power of budding has apparently never been acquired. If we compare the larvae of the rest of the Ascidiacea, termed the **Ascidiae compositae,** with the larvae of the Ascidiae simplices, we find that the former are distinguished from the latter by the precocious appearance of the peculiar features of the adult. In other words, the Ascidiae simplices preserve the larval stage in its most unmodified form, and it is to them, therefore, that we turn in order to select a type for special description.

CYNTHIA

The most recent work on the subject is by Conklin (1905), who has followed the development of *Cynthia partita,* cell for cell, until the attainment of the larval form. He has done the same for *Ciona intestinalis,* and for *Phallusia* (1911), and his researches have made it probable that the same general scheme applies to all the Ascidiae simplices. What is related for *Cynthia* will hold true, with slight modifications, for the common simple Ascidians on the coasts of England, and the work of Van Beneden and Julin (1884) makes it clear that the development of *Clavelina,* the simplest form of the Ascidiae compositae, follows the same general course, although, even here, the telescoping of development, characteristic of compound Ascidians, has begun to appear.

Cynthia partita is an Ascidian found in abundance at Wood's Hole. It lives fairly well in laboratory tanks, and it lays eggs in the evening, which, in the course of about ten hours, develop into fully organized free-swimming larvae. In order, therefore, to be able to study the development by daylight, Conklin extracted eggs from the ovary and artificially fertilized them in the morning. Of such artificially fertilized eggs few developed, but these few were sufficient to enable the development to be thoroughly investigated. In the case of *Ciona intestinalis,* on the contrary, it is quite easy to artificially fertilize the eggs if the sperm from another individual be used. The animal's own sperm will not fertilize its eggs.

The eggs of *Cynthia* were examined living, on a slide, in sea-water, covered with a coverslip supported by fragments of coverslip about $\frac{1}{5}$ mm. thick. Under these circumstances the eggs could be rolled about and examined from all sides. When it was desired to make whole mounts, Kleinenberg's picro-sulphuric acid gave the best results. The best method of staining was to use both Delafield's haematoxylin and picric acid. The eggs are first stained with haematoxylin, and this is differentiated in the usual way by adding acid alcohol; then for dehydration the alcohols of 90 per cent and absolute strength, made yellow by the addition of picric acid crystals, are used. In this way the mass of the egg is stained yellow, but the nuclei are stained blue. For sections it was found best to preserve the eggs in picro-acetic acid. Such material can be stained with haematoxylin followed by eosin dissolved in oil of cloves, on the slide.

Conklin commenced his study of the development with the ovarian egg. In the young oocyte there is no yolk, and the nucleus occupies a nearly central position in the egg. On the one side of the nucleus is a rounded mass which stains deeply with eosin. This Conklin considers to be the "attraction sphere," which functioned in the cell division which gave rise to the oocyte; it now functions as **yolk-nucleus**, *i.e.* the deposition of yolk-granules takes place round it as a centre. A layer of peripheral cytoplasm remains free from yolk; in this, however, yellow pigment granules are deposited. The yolk spherules are of a slaty-grey colour. There is an enormous vesicular nucleus (germinal vesicle), with a large nucleolus, and near the nuclear membrane a few chromatin granules.

As the oocyte grows it is surrounded by the so-called **test cells**. These are simply follicle cells or rudimentary ova; round the nucleus of each there is the same collection of yellow granules as are found in the peripheral layer of the ovum. These "test cells" become actually embedded in the peripheral layer of the oocyte whilst it is growing, but when it is ripe and discharged from the ovary the test cells are expelled from the cytoplasm, and a **chorion** is formed. Between the chorion and the egg there is a space formed by the shrinkage of the latter, the perivitelline space. In this the expelled test cells lie.

After laying, the egg begins to undergo maturation changes. The nuclear membrane dissolves and so does the large nucleolus. *The clear nuclear sap flows upwards and forms a cap of clear cytoplasm, lying at*

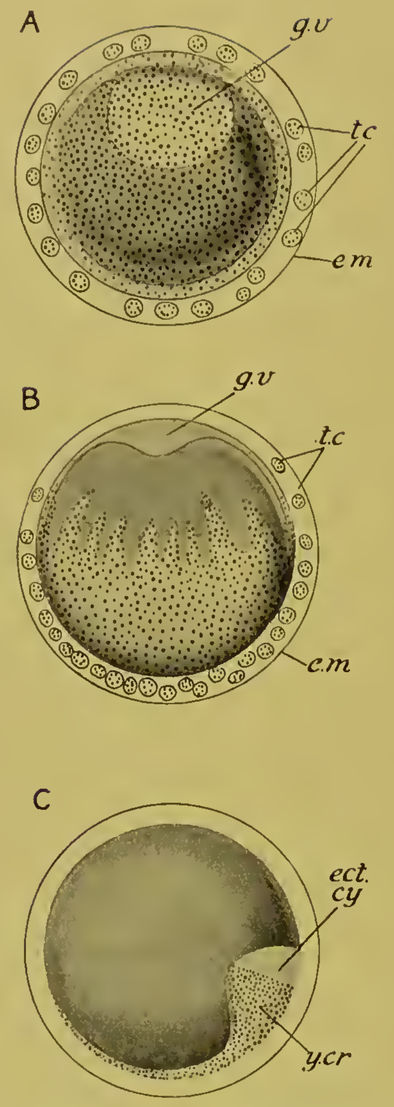

FIG. 442.—The egg of *Cynthia partita* before and during fertilization. (After Conklin.)

[The yellow cytoplasm is indicated by dotting the dark-blue cytoplasm by dark shading.]

A, egg before fertilization with large germinal vesicle. B, egg immediately after fertilization, showing the down-streaming of the yellow cytoplasm and the outflow of the contents of the germinal vesicle. C, egg a little later, showing the aggregation of the yellow cytoplasm to form the yellow crescent at the posterior side of the egg. *ect.cy*, clear cytoplasm which will give rise to ectoderm; *e.m*, egg-membrane; *g.v*, germinal vesicle; *t.c*, test cells; *y.cr*, yellow crescent.

what will be the animal pole of the egg. In the centre of this cap lie the chromosomes which are connected together by a faintly granular substance. Out of this granular substance the first maturation spindle is developed, the spindle fibres appearing as rods of granules. No further development takes place unless the egg be fertilized.

The spermatozoon is able to burrow through the chorion; it enters the cytoplasm at the vegetative pole of the egg, as in the case of the egg of *Amphioxus.* Immediately after its entry the division initiated by the maturation spindle is completed, and the first polar body is given off. This consists of clear cytoplasm and does not divide. A new spindle is reconstructed out of the remains of the old one, and a new division occurs by which the second polar body is given off. The second polar body is in all respects similar to the first; both bodies remain attached to the egg, in many cases throughout the whole of the embryonic development, and so the position of the animal pole can be accurately determined. In neither maturation division is there a trace of a centrosome.

Whilst the maturation divisions have been proceeding, cytoplasmic movements of a violent character have been taking place. Both the clear cytoplasm and the clear peripheral layer with the yellow granules flow downwards to the vegetable pole of the egg, and form a cap there which surrounds the sperm head. With such violence does this flow take place that the "test cells" are often carried along with it, and are found aggregated in a heap at the vegetable pole (Fig. 442, B). At the animal pole is left only a small mass of clear substance surrounding the maturation spindle. The slaty yolk is now massed at the animal pole. At the vegetable pole the clear protoplasm is superficial to and more extensive than the yellow substance.

The spermatozoon at first moves towards the centre of the egg from whatever point it enters, until it has traversed the clear and the yellow protoplasm and reached the yolk. This is its **penetration path.** Then it changes its direction and moves towards the posterior end of the egg. This is its **copulation path.** As it thus moves it seems to draw with it a large part of the yellow protoplasm, which forms a crescent just below the equator of the egg, with the two arms reaching half-way round; the centre of this crescent is the lower pole of the egg. The clear cytoplasm moves towards the posterior side of the egg, around and outside the yellow substance (Fig. 442, C).

The male and female pronuclei meet near the lower pole of the egg; since the female pronucleus, after giving off the second polar body, descends to meet the male. The zygote nucleus then moves inwards towards the egg's centre. The male pronucleus, before uniting with the female pronucleus, has developed at one side a well-marked **aster.** Before the zygote-nucleus divides, this aster divides into two parts, placed one at each end of the first spindle; so that, although in the maturation divisions no asters are found, in all the divisions which occur during the development of the egg asters are formed. As the zygote-nucleus moves inwards it draws with it the clear cytoplasm, leaving most of the yellow cytoplasm behind. Some clear cytoplasm, however, is left at the surface as a narrow band above the yellow crescent, and some yellow cytoplasm moves inwards. The egg is now ready to undergo the first cleavage.

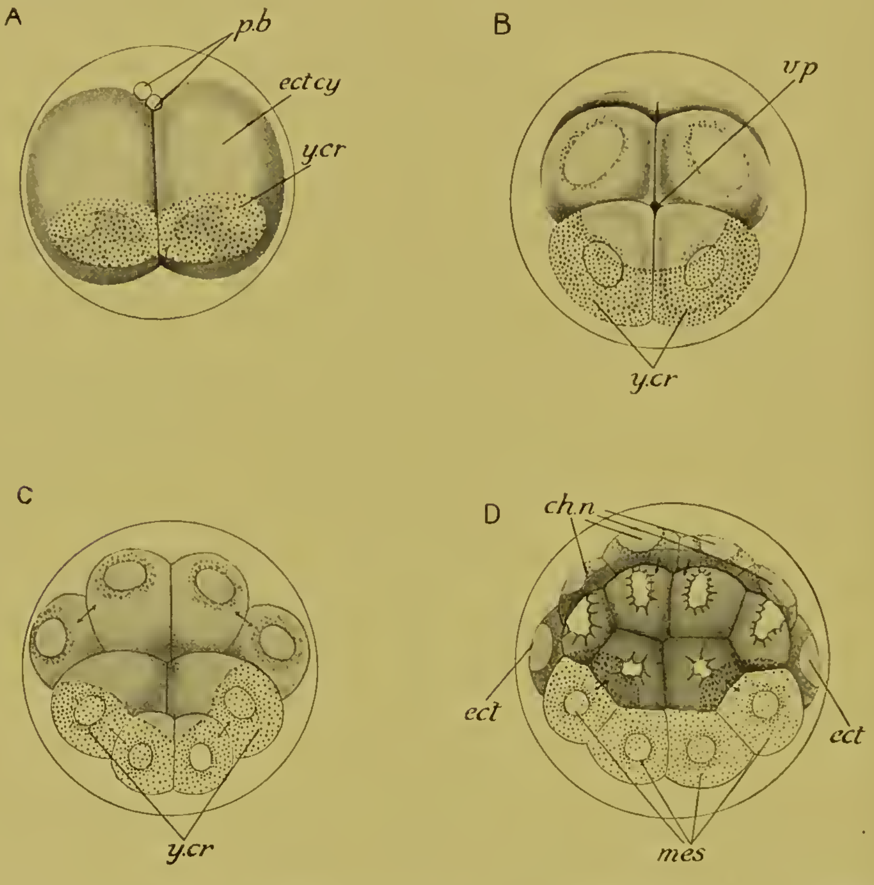

Fig. 443.—Stages in the segmentation of the egg of *Cynthia partita*. (After Conklin.)

[The different coloured cytoplasms are indicated as in the preceding figure.]

A, 2-cell stage viewed from behind. B, 8-cell stage viewed from the vegetative pole. C, 16-cell stage viewed from the vegetative pole. D, segmenting egg passing into the 32-cell stage, viewed from the vegetative pole. *ch.n*, chorda-neural cells; *ect*, ectodermal cells; *ect.cy*, cytoplasm which will give rise to the ectoderm; *mes*, mesodermal cell; *p.b*, polar bodies; *v.p*, vegetative pole; *y.cr*, yellow crescent.

The first cleavage divides the egg into two precisely equal cells; the yellow crescent is also divided into two equal parts, and into each crescent, after division, an intrusion of grey yolk takes place, which indents it (Fig. 443, A). The two sides of the indentation join round the intrusion, and the crescent is thus re-established. In this way the yellow substance receives accessions from the yolk. After division the nuclei, with the clear substance surrounding them, rotate round the egg towards the animal pole. The yolk is thus displaced from this pole, which, although it was temporarily the seat of the most of the yolk, remains ever afterwards relatively free from yolk and becomes the seat of an accumulation of clear cytoplasm; although even here a thin external layer of cytoplasm, with yellow yolk-grains, is found.

At the next cleavage the two cells become four; the two anterior cells are slightly larger than the two posterior, which receive all the yellow substance. The four cells then become eight in the usual way, by dividing into two tiers, and the four cells at the animal pole are smaller than the four at the vegetable and contain all the clear cytoplasm. There is a cross furrow observable when eggs in this stage are viewed from the side. This arises from the circumstance that the furrows separating upper and lower cells come to slant downwards and forwards, and consequently they strike the vertical furrow dividing anterior from posterior cells in different places. The anterior vegetative cells are the largest, and the anterior animal cells the smallest in the egg.

We may here anticipate the final result of the development so far as to say, that the animal pole of the egg corresponds roughly to the antero-ventral region of the embryo, whilst the vegetative pole corresponds to the postero-dorsal region of the embryo. If the reader confuses "animal" and "dorsal" regions of the egg, it will be impossible to follow the account intelligently.

When the next cleavage occurs the spindles of all the dividing nuclei are parallel to the horizontal plane, but are oblique to the vertical plane of the egg. The 16-cell stage (Fig. 443, C) consists of two tiers of eight cells. The animal hemisphere, in consequence of the direction of the spindles of the dividing cells, is narrow in front and broad behind, whereas the vegetative hemisphere is broad in front and narrow behind, and here the two smallest cells in the egg are delimited; these consist almost entirely of yellow substance and constitute a valuable landmark. The two cells immediately in front of these contain the rest of the yellow substance, but in addition a great deal of yolk. The remaining four of the vegetative cells contain principally the slaty-blue yolk, but have a certain amount of clear substance near their anterior borders.

The next cleavage period raises the number of cells to thirty-two. On the vegetative side of the egg the purely yellow cells divide so as to form four, lying side by side. The half-yellow, half-blue cells divide *so as to separate the yellow and blue portions*; and the yellow portions lie to the outside, so that there is now a crescent, concave in front, of six yellow cells, running across this hemisphere. The anterior, wholly blue cells, of the vegetative hemisphere, divide into anterior and posterior daughters; the anterior daughters lying lower than their sisters. There result two crescents of four cells each, the front one lying just beneath the equator of the egg, and the posterior running across the middle of the back. The cells of the posterior crescent contain only slaty-blue, yolky substance, but those of the anterior crescent contain a rim of clear substance in front and slaty-blue behind. The cells of the animal region of the egg, corresponding to the ventral hemisphere of the embryo, divide into similar daughters, all alike; in front, however, the two most anterior just surmount the equator. They all consist of clear substance and yolky material, but the clear substance is much more prominent than the yolk. At this stage, too, they are all columnar, and the cells of the vegetative pole broad and flat; at a later period, as we shall see, the shapes of the cells of the two poles become interchanged. The cells have separated from one another internally so as to give rise to a **blastocoele,** and the egg has now become a **blastula** (Fig. 445, A).

It is now possible to see clearly the exact regions of the embryo. The cells of the animal hemisphere, or antero-ventral portion of the embryo, give rise to ectoderm. Those two, however, which surmount the equator in front when the egg is viewed from the dorsal aspect, *i.e.* from the vegetative side, give rise to the anterior portion of the **neural plate.** The blue cells of the vegetative hemisphere give rise to the endoderm, but the most anterior cells of this hemisphere, consisting of clear and blue substances, form the **dorsal lip of the blastopore,** corresponding to the edge x in *Amphioxus.* They are called **chorda-neural** cells (*ch.n,* Fig. 443, D) by Conklin, because, like the cells forming that edge in the embryo of Amphioxus, *they give rise by further division to both neural plate cells (ectoderm) and notochordal cells (endoderm), the former being constituted by the clear and the latter by the blue portions of the cells.* The transverse band of yellow cells gives rise not only to the longitudinal muscles of the tail of the larva but also to what Conklin calls "**mesenchyme,**" *i.e.* anterior mesoderm to which the muscles and genital organs of the adult owe their origin.

A register of the whole thirty-two cells shows, therefore, fourteen ventral ectoderm cells, two neural plate cells, four chorda-neural cells, six endoderm cells, and six yellow mesoderm cells. The blue cells form a broad band in front which gives rise to the **pharynx** of the larva, and to a tongue, projecting backwards between the horns of the yellow crescent, which is the rudiment of the **tail endoderm.**

By the next cleavage sixty-four cells are produced. Turning first to the animal hemisphere, we find that the spindles of the dividing cells are directed in various ways, but the result is to produce a coherent sheet of twenty-eight similar ectoderm cells. The neural cells divide so as to produce a curved transverse line of four cells, and to the outer edges of this crescent there is added, on either side, a cell from the rest of the ectoderm which also contributes to the formation of the nervous system; so that, in this way, a crescent of six neural cells is formed.

Coming now to the vegetative hemisphere, we find that the chorda-neural cells have divided into anterior and posterior daughters. The four anterior are also neural cells and enter into the formation of the neural plate. The four posterior are the rudiment of the **notochord** (Fig. 444, A). The blue endoderm cells divide chiefly into anterior and posterior cells, but in front the lateral endoderm cell on either side divides into an inner and an outer daughter cell. The outer cell on either side contains less yolk than the inner, and is the so-called **anterior mesenchyme cell**, which is the rudiment of the most anterior part of the mesoderm.

Of the six yellow cells, the two outer ones on either side divide into dorsal and ventral components. The dorsal component contains less yellow substance than the ventral, and appears of a paler colour and gives rise to mesenchyme. The inner cell on each side divides into anterior and posterior daughters; the anterior daughters with the ventral daughters of the outer cells form the rudiment of the **longitudinal muscles** of the tail of the larva, while the posterior daughters of these inner cells form mesenchyme and constitute the **posterior mesenchyme cell** on each side. The divisions of the cells of the vegetative hemisphere take place first, *and these cells pass into a state of rest, during which they alter their form and become columnar, whilst the cells of the animal hemisphere are dividing. These latter, on the contrary, now cease to be columnar and become flat* (Fig. 445).

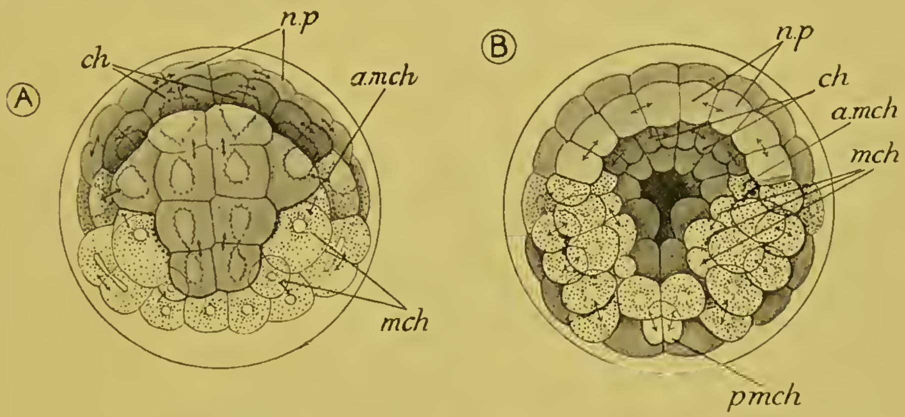

FIG. 444.—Illustrating the gastrulation of the egg of *Cynthia partita*. (After Conklin.)

[The coloured cytoplasms are indicated as in the two previous figures.]

Both figures represent eggs viewed from the vegetative pole, *i.e.* from above and behind.

A, stage in which the semicircular dorsal lip of the blastopore is just beginning to form. B, stage in which the invagination of the endoderm is well advanced. *a.mch*, anterior mesenchyme cell; *ch*, notochordal cells; *mch*, mesenchyme cells; *n.p*, neural plate cells; *p.mch*, posterior mesenchyme cell. The dotted cells which are not mesenchyme are mesodermal cells.

If we make a register of the whole sixty-four cells, we find: animal hemisphere, twenty-six ordinary ectoderm cells, six neural cells; vegetative hemisphere, four neural cells, four notochordal cells, two anterior mesenchyme cells, four mesenchyme cells, two posterior mesenchyme cells, six muscle cells, and ten endoderm cells.

After this cleavage we can no longer speak of a general period of cleavage affecting all the cells of the egg, because one set of cells will have passed into a new period of cleavage before another set have completed the previous cleavage. The crescents of cells, neural and notochordal, derived from the posterior lip of the blastopore, increase to eight cells each. The two front muscle cells on each side are increased to four, whilst the mesenchyme cells above them also divide.

The **gastrulation** now begins, as in Amphioxus, by an inwardly directed cytotaxis of the endoderm cells. The beginning of this is no doubt really the change in shape that, as we have just noted, the cells of the vegetative hemisphere have undergone, namely, from a flat to a columnar shape. This change leads to a contraction of their surface area, whilst the contrary change in shape undergone by the ectoderm cells increases their area.

At this stage in the proceedings there is, in front, a semicircular arc, opening backwards, of eight chordal cells; and behind a semicircular arc, opening forwards, of twelve mesenchyme cells. These two arcs taken together form a complete circle inside which are the endoderm cells. Beneath the mesenchymal arc lies an arc of six muscle cells, whilst outside the chordal arc is an arc of eight neural cells. If, however, as Castle (1894) maintains, the first cell of the mesenchymal arc on each side enters into the formation of the notochord, then the circle described above will consist of ten notochordal cells in front and of ten mesenchymal cells behind.

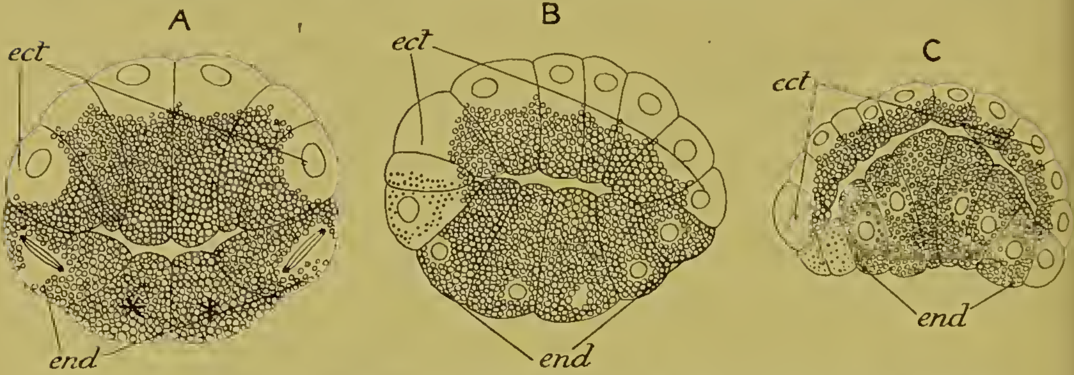

FIG. 445.—Sections through eggs of *Cynthia partita*, in order to illustrate the changes in form undergone by ectodermal and endodermal cells during the process of gastrulation. (After Conklin.)

The small circles in the cells represent yolk-granules.

A, transverse section through egg with about forty cells, showing columnar ectoderm and quadrate endoderm. B, longitudinal sagittal section through egg with sixty-four cells, showing endoderm and ectoderm cells both columnar. C, longitudinal sagittal section through egg with one hundred and ten cells, showing flattened ectoderm cells and columnar endoderm cells. *ect*, ectoderm cells; *end*, endoderm cells.

At a stage a little older than that shown in Fig. 444, B, the neural arc described above begins to grow back, overarching the chordal arc and the endoderm cells. Soon afterwards the ectoderm cells lying outside the mesenchyme ring increase in number and become involved in this overarching process. It follows that whereas the outline of the lip of the gastrula, viewed from the vegetative surface, appears semicircular, the blastopore is really wide in front and narrow behind; for behind it is a groove between the two columns of muscle and mesenchyme cells on each side, the mesenchyme cells being above and external as regards the muscle cells.

The overarching rim of cells, which is the anterior lip of the blastopore and corresponds to the region marked *x* in the gastrula of *Amphioxus*, continues its growth backward till it covers three-quarters of the dorsal face of the embryo. Then the groove between the lateral walls of muscle and mesenchyme becomes tilted upwards till it becomes vertical, and the mesenchyme cells are placed in front of the muscle cells. This change is due to the sudden upgrowth of the posterior and ventral lip of the blastopore, the region corresponding to the region *y* in the gastrula of *Amphioxus*. The

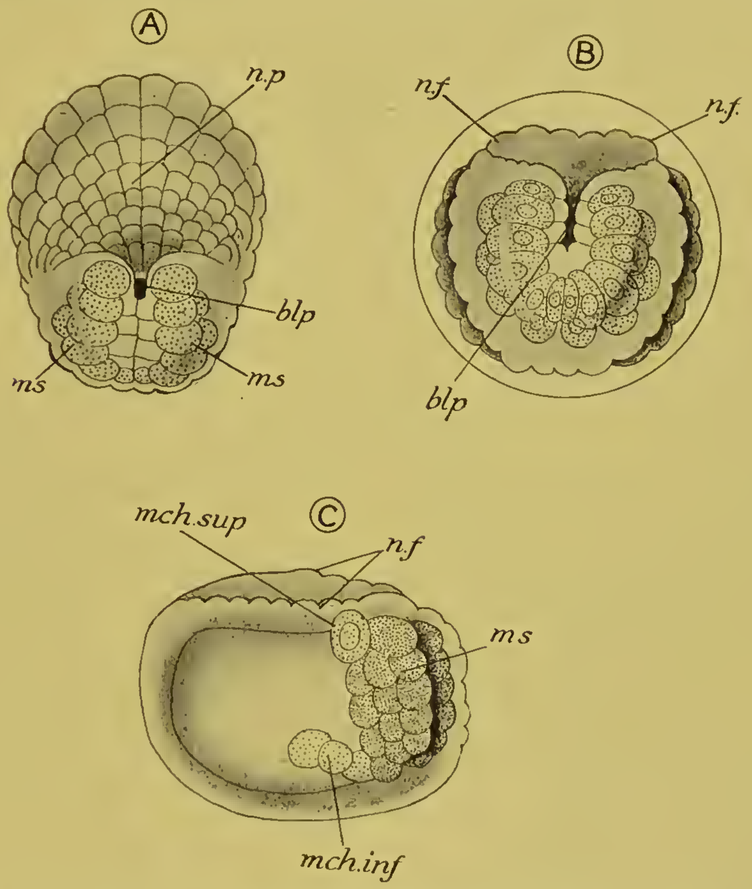

FIG. 446.—Illustrating the closure of the blastopore in the gastrula of *Cynthia partita*. (After Conklin.)

A, dorsal view of advanced gastrula, showing the blastopore as a minute pore at the hinder end of the neural plate. B, posterior view of advanced gastrula, showing the neural folds. C, lateral view of advanced gastrula, showing the rotation of the mesodermal bands into a vertical position by the upgrowth of the ventral lip of the blastopore and the division of the mesenchyme into a superior and an inferior mass. *blp*, disappearing blastopore; *mch.inf*, inferior mass of mesenchyme; *mch.sup*, superior mass of mesenchyme; *ms*, mesodermal band; *n.f*, neural fold; *n.p*, neural plate.

blastopore, instead of being directed posteriorly, becomes directed dorsally (Fig. 447); in fact, the change which occurs is precisely parallel to the change which takes place during the closure of the blastopore in *Amphioxus*.

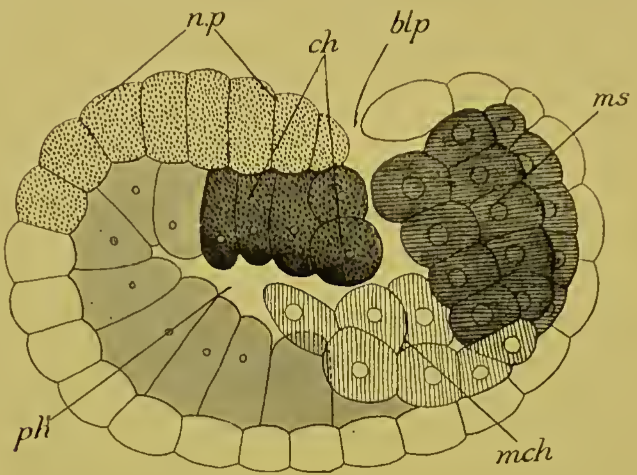

FIG. 447.—Optical sagittal section of gastrula of *Cynthia partita*. (After Conklin.)

blp, blastopore; *ch*, notochord; *mch*, mesenchyme; *ms*, mesodermal band in vertical position; *n.p*, neural plate; *ph*, pharynx.

The column of mesenchyme cells then becomes broken into two masses, an anterior one adhering to the top of the column of muscle cells, and a posterior one attached to the foot of this column.

Soon afterwards the vertical columns of muscle cells are again forced into a horizontal position, and eventually constitute two rows of muscle cells, one above the other, on each side of the embryo. Each row consists of about six cells, and each cell forms a long, horizontal lozenge-shaped structure, and develops muscular fibrils in its substance. This change, from a vertical to a horizontal position, which occurs in the muscle cells, is due to the growth in length of the hinder end of the embryo, a process which leads to the formation of the **tail.** The anterior mesenchyme is now situated in front of the rows of muscle cells, and is incorporated in the trunk

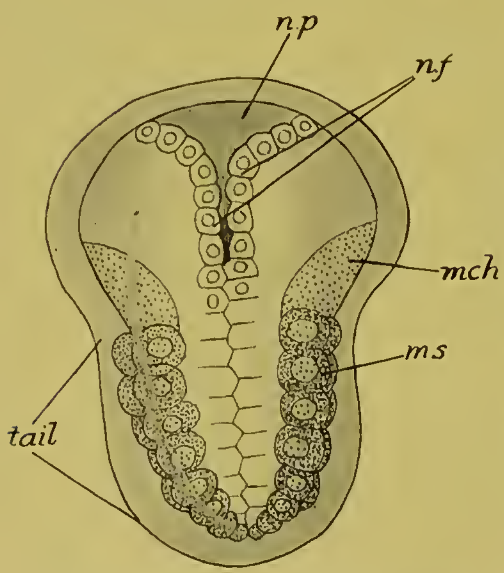

FIG. 448.—Dorsal view of an embryo of *Cynthia partita* after the closure of the blastopore, in order to show the formation of the tail. (After Conklin.)

mch, mesenchyme which will give rise to the mesoderm of the adult Ascidian; *ms*, mesodermal band drawn out into a horizontal position by the growth of the tail; *n.f*, neural folds; *n.p*, neural plate.

of the embryo (Fig. 448); it lies on each side of the pharyngeal wall, wedged in between this and the ectoderm (Fig. 449). The posterior mesenchyme is situated at the hinder end of the tail (Fig. 450).

When the blastopore has been reduced to a pore on the dorsal surface, the **neural folds** (often termed the **medullary folds**) make their appearance (*n.f*, Fig. 446). These are a pair of folds of ectoderm arising from the sides of the neural plate, meeting each other in the middle line behind the blastopore, but diverging from one another widely in front. As development proceeds they meet each other in the middle line farther and farther forward; thus the blastopore is covered in, and, as in *Amphioxus*, a **neurenteric canal** is formed. Then the neural plate is covered in and converted into a **neural tube** covered by ectoderm.

The neural plate is wide in front and gives rise to a correspondingly wide section of the neural tube; this is the **brain-vesicle**; the remainder of the neural tube is narrow and constitutes the **spinal cord.** For a short time the front part of the brain-vesicle communicates with the exterior by a pore termed the **neuropore,** corresponding to the neuropore of the larva of *Amphioxus*, and, like it, due to an imperfect union of the neural folds in front. The neuropore, however, soon closes (Willey, 1893), but reopens at the close of larval life when the larva undergoes metamorphosis.

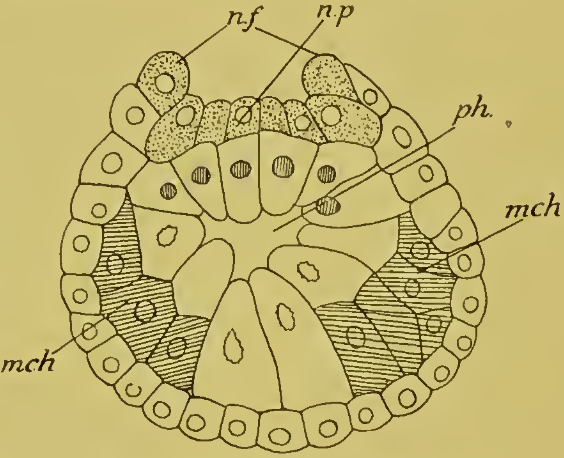

FIG. 449.—Transverse section through the middle of a gastrula of *Cynthia partita.* (After Conklin.)

mch, mesenchyme lying at the sides of the gut; *n.f*, neural fold; *n.p*, neural plate; *ph*, cavity of pharynx.

The larva soon afterwards bursts its chorion and enters on its free-swimming career, propelling itself like a fish by lateral blows of its tail. For a complete exposition of its organisation (which is taken for granted by Conklin) we must turn to the older work of Kowalevsky (1866, 1871), who worked chiefly with *Ciona intestinalis* and with *Phallusia mammillata.*

In the fully developed larva a **mouth** is formed by a small insinking of the ectoderm in front of the nerve tube. This insinking involves the spot where the neuropore was situated, so that the brain-vesicle would open into the pharynx if the neuropore were still open. This is the case in *Clavelina* (Willey, 1893), but in simple Ascidians, as we have seen, the neuropore becomes closed during free-swimming life, but reopens during metamorphosis. The hinder end of the endoderm forms a solid cord underlying the notochord; it soon breaks up into wandering cells. On the dorso-lateral surfaces of the trunk a pair of invaginations make their appearance. These are the **atrial invaginations**; they meet and fuse each with two evaginations of the pharynx, and thus are formed the first two **pairs of gill-slits**, which, for reasons to be given later, we designate as gill-slit No. 1 and gill-slit No. 4.

From the hinder wall of the pharynx there grows out a tube-like diverticulum, the rudiment of the adult **intestine**. The end of this curves up the left side of the body, and in the larvae of some species even comes into contact with the ectoderm, not far from the mid-dorsal line; but no anus is formed till the free-swimming life is over. Along the ventral wall of the pharynx there is differentiated a broad strip of columnar cells; this is the **endostyle**.

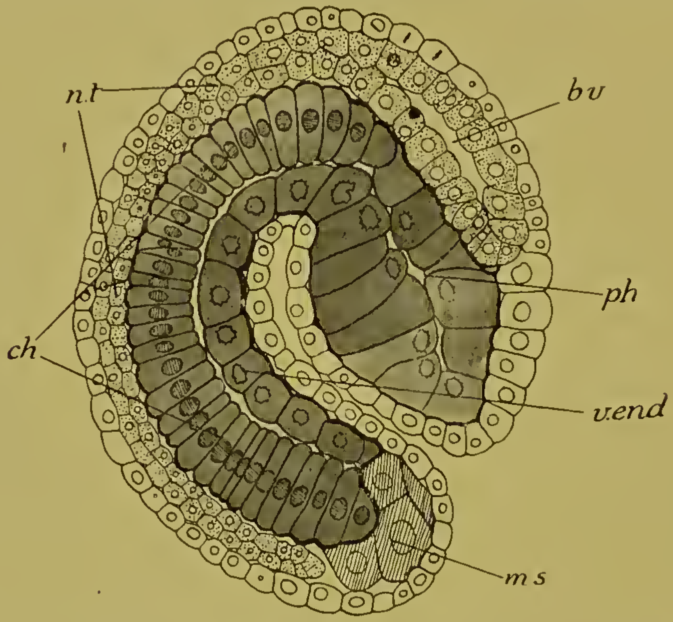

FIG. 450.—Longitudinal sagittal section of tadpole of *Cynthia partita* just before it escapes from the egg-membrane. (After Conklin.)

b.v, brain-vesicle; *ch*, notochord; *ms*, mesodermal band (seen by slight obliquity of the posterior end of the section); *n.t*, nerve tube; *ph*, pharynx; *v.end*, ventral cord of endoderm cells in the tail.

Finally, the wall of the brain-vesicle undergoes modification. On the right side a hemispherical outgrowth takes place, and the cavity of this cup becomes filled up with a secretion which becomes pigmented. This is the **cerebral eye** of the Ascidian tadpole, and corresponds, without doubt, to one of the two optic vesicles of the brain of a higher Vertebrate. Close behind the eye rudiment there is another pigmented area of epithelium which is the rudiment of the **ear** or **balancing organ**. It is soon separated from the eye and forced round to the floor of the brain-vesicle by an expansion of the epithelium between it and the eye, which thus gives rise to the thin-walled epithelium forming the greater part of the wall of the brain-vesicle. The ear eventually forms a median prominence, shaped like

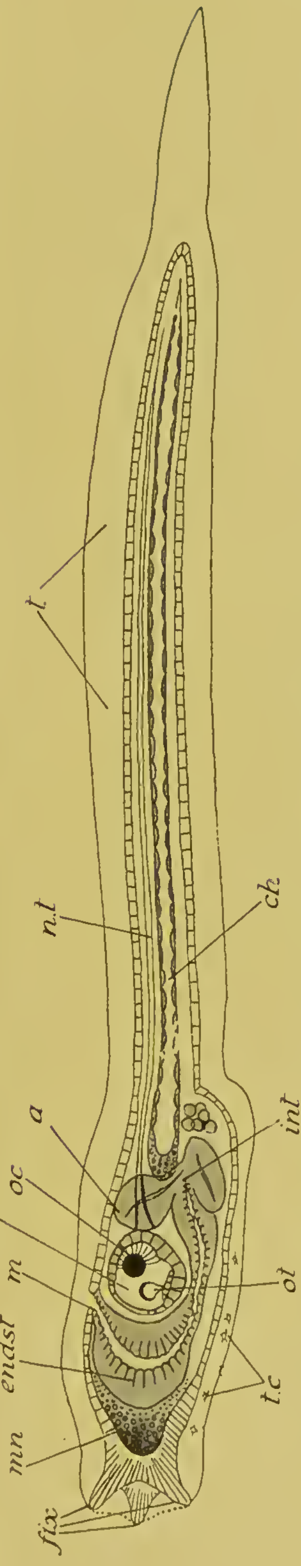

FIG. 451.—Free-swimming tadpole of the simple Ascidian *Ciona intestinalis* seen from the side. (Combined from two figures by Kowalevsky.)

a, position where anus will be formed; *b.v*, brain-vesicle; *ch*, notochord; *endst*, endostyle; *fix*, fixing processes; *int*, rudiment of intestine; *m*, mouth; *mn*, chin (mental process); *n.t*, nerve-tube; *oc*, eye-cup; *ot*, otolith; *t*, test; *t.c*, test cells.

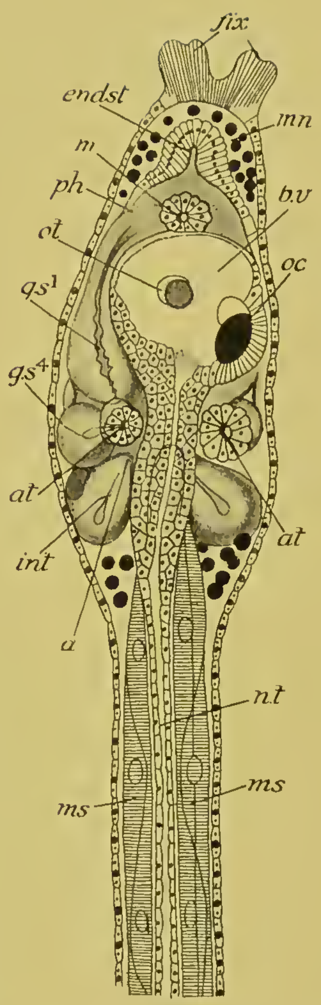

FIG. 452.—Anterior portion of free-swimming tadpole of the simple Ascidian *Phallusia mammillata*, seen from above. (After Kowalevsky.)

a, position where anus will be formed; *at*, atrial involutions; *b.v*, brain-vesicle; *endst*, endostyle; *fix*, fixing processes; gs^1, gs^4, the first two gill-slits; *int*, intestine; *m*, mouth; *mn*, chin (mental process); *ms*, longitudinal muscles of the tail; *n.t*, nerve tube; *oc*, eye-cup; *ot*, otolith; *ph*, pharynx.

a cup on a stalk, carrying in its apex a spherical pigmented concretion, the otolith. The hinder wall of the brain-vesicle is thick, and forms a ganglion, termed the **visceral ganglion** (*gang.visc*, Fig. 454, F). There is a protuberant region which we may term the **chin** (*mn*, Fig. 451). Below the mouth the ectoderm develops three papillae of glandular cells, one median and two lateral, which are the **organs of fixation.**

Although the larva has a mouth it cannot feed, because this is closed by a gelatinous cuticle termed the **test** or **mantle.** The test is secreted by the ectoderm and enswathes the whole body, covering over both mouth and atrial openings. It contains certain cells called **test cells,** which are budded from the ectoderm (*t.c*, Fig. 451), but these cells are to be carefully distinguished from the cells bearing the same name which surround the egg; these latter, as we have seen, are rudimentary ova.

After a short free life the larva fixes itself to the substratum by the papillae on its chin, and then undergoes a rapid **degenerative metamorphosis.** By the contraction of the ectoderm of the tail, the notochord, nerve cord, and muscles are bent and broken into fragments, which are rapidly devoured by wandering cells (Fig. 453). The ectoderm becomes invaginated so as to form pockets, and these pieces of intucked ectoderm are attacked and devoured by wandering cells; and by these processes the whole of the tail is disposed of. The chin region grows enormously, and by this growth the mouth is carried upwards, away from the substratum to which the animal is attached. This process is similar to the one by which the mouth is rotated upwards in the metamorphosing larvae of Cirripedia, Polyzoa, Entoprocta, and Crinoidea; and it occurs for the same reason in all four cases, viz. from the physiological necessity of lifting the mouth up so as to put it into a better position for catching floating plankton.

Willey (1893) has followed in some detail the metamorphosis of *Ciona intestinalis,* and his results have been confirmed by other workers. He finds, as already mentioned, that when the metamorphosis begins the neuropore is reopened; and he suggests, what is *a priori* extremely likely, that this opening has persisted, in a potential form, as union of the ectodermic wall of the brain-vesicle with the ectoderm all through the larval life. During larval life a gutter, which we may term the **hypophysial canal** (*hyp*, Fig. 454), is formed in the dorsal wall of the brain-vesicle. This gutter becomes completely grooved off from the vesicle behind, where it is continuous with the cavity of the spinal cord, but opens into the vesicle in front.

At the beginning of metamorphosis, when the neuropore is reopened, the hypophysial canal becomes continuous with this pore, and the rest of the brain-vesicle becomes completely grooved off from the canal. The brain-vesicle then rapidly degenerates (Fig. 455), and the sense-organs are broken up and absorbed. The **adult ganglion** is formed as a new proliferation (Figs. 454, 455) from the

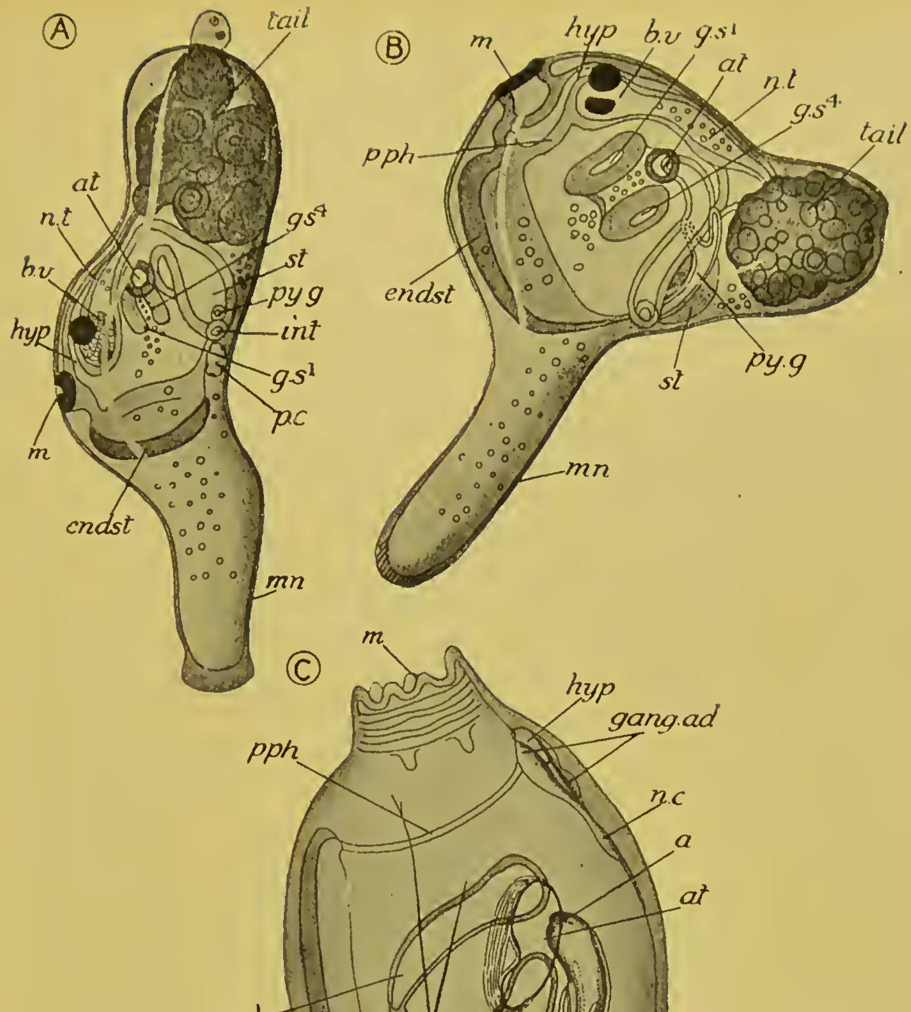

Fig. 453.—Illustrating the metamorphosis of the tadpole of *Ciona intestinalis*. (After Willey.)

A, young tadpole just after fixation, seen from the right side—the mouth is still in the larval position and the tail is just beginning to degenerate. B, young fixed *Ciona*, in which the mouth is rotated upwards and the tail is reduced to a granular mass. C, young fixed *Ciona*, in which the mouth has attained the upper pole of the animal and in which the tail has disappeared. *a*, anus; *at*, atrial canal; *b.v*, brain-vesicle; *endst*, endostyle; *gang.ad*, adult ganglion; *g.s*$^{1-4}$, the first four gill-slits; *hyp*, hypophysial canal; *int*, intestine; *m*, mouth; *mn*, chin (mental process); *n.c*, nerve cord; *n.t*, nerve tube; *oes*, oesophagus; *pc*, pericardial sac; *pph*, peripharyngeal ciliated band; *py.g*, pyloric gland; *st*, stomach.

dorsal wall of the hypophysial canal; the visceral ganglion of the larva, which formed the thick posterior wall of the brain-vesicle,

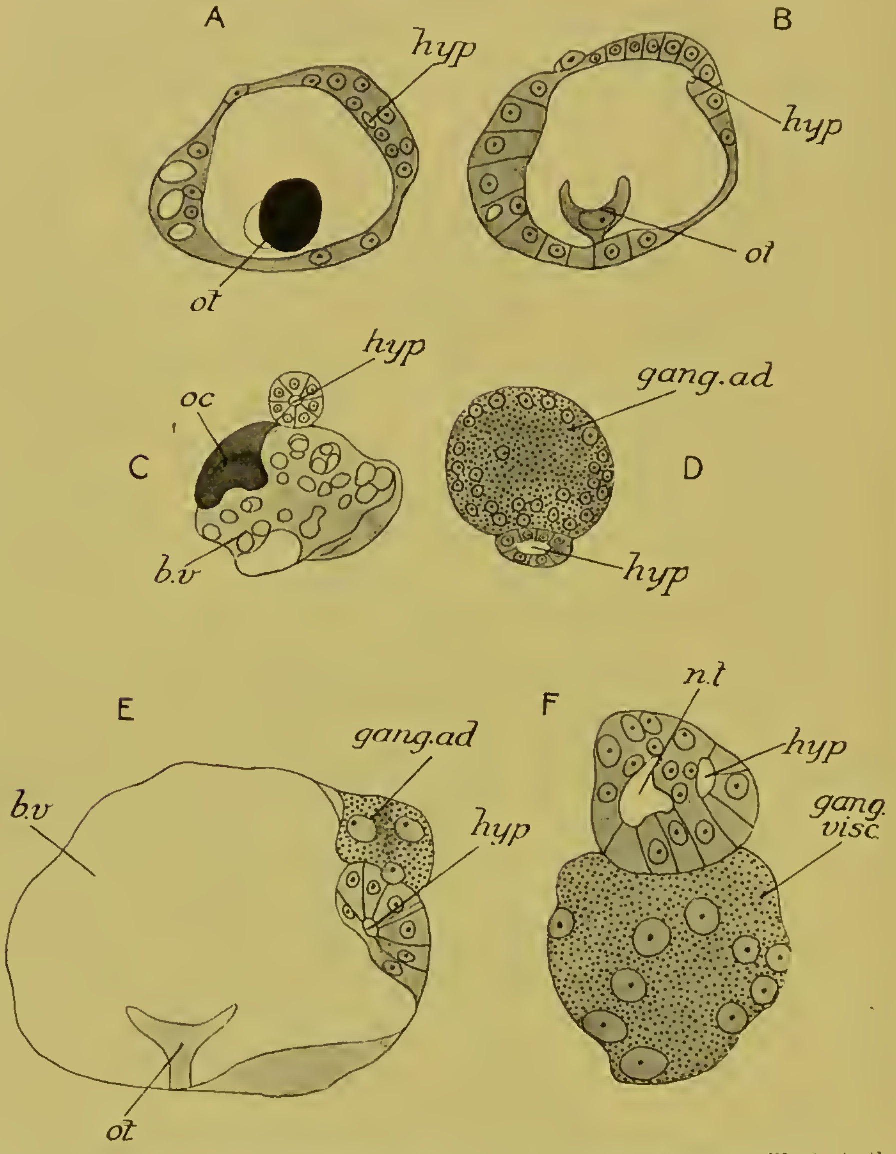

FIG. 454.—Transverse sections through the brains of Ascidian tadpoles to illustrate the development of the hypophysial tube and of the adult ganglion. (After Willey.)

A, B, sections through brain of a newly-hatched tadpole of *Ciona intestinalis*: A, the more anterior section, shows the pigmented otolith and the hypophysial canal running in the wall of the brain-vesicle; B, the more posterior section, shows the hypophysial canal opening into the brain-vesicle and the cup-shaped support for the otolith. C, section through the brain of a just-fixed tadpole of *Ciona intestinalis*, showing the degenerating larval brain and the hypophysial tube above it. D, section through the brain of a young *Ciona intestinalis*, showing the adult ganglion as a new formation on the dorsal wall of the hypophysial tube. E, F, sections through the brain of a tadpole of *Clavelina lepadiformis*: E, the more anterior section, shows the brain-vesicle and the origin of the hypophysial tube and of the adult ganglion from its wall; F, the more posterior section, shows the cavities of the nerve tube and of the hypophysial tube and the larva visceral ganglion. *b.v*, brain-vesicle; *gang.ad*, rudiment of adult ganglion; *gang.visc*, visceral ganglion; *hyp*, hypophysial tube; *n.t*, nerve tube; *oc*, degenerating remnant of the larval eye; *ot*, otolith and its support.

degenerates and disappears. The greater part of the spinal cord is lost with the degeneration of the tail, but the small portion which remains loses its cavity; whilst from the posterior end of the hypophysial canal glandular pockets are formed, which give rise to the **subneural gland** of the adult, and which have been compared to the pituitary body of the higher Vertebrate.

The intestine, which in *Ciona* is less developed in the free-swimming larva than is the case in some other species of simple

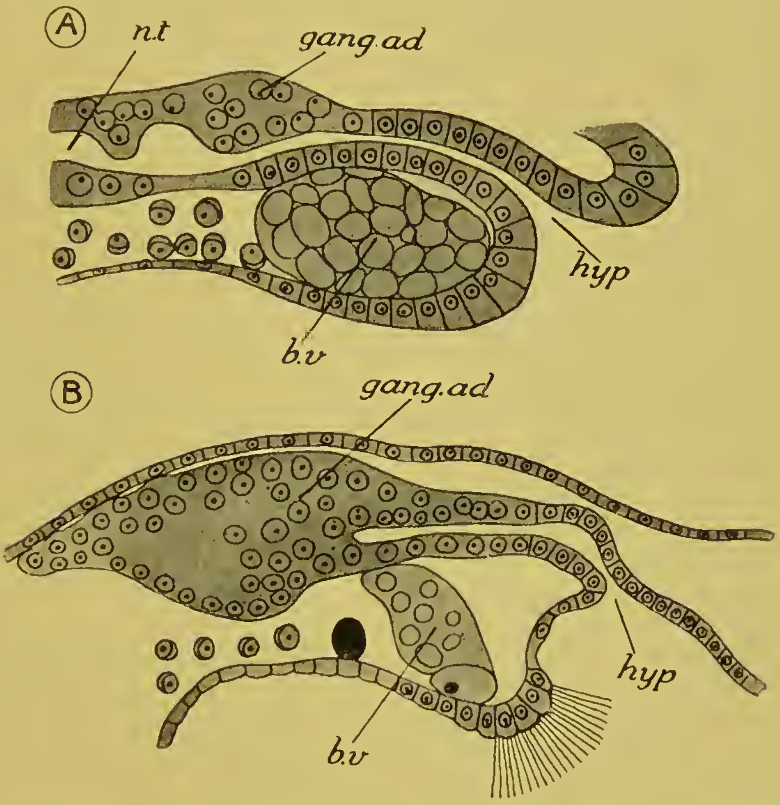

Fig. 455.—Longitudinal sagittal sections through the brain-vesicles and hypophysial tubes of just-fixed tadpoles of *Ciona intestinalis*. (After Willey.)

A, earlier stage; note that the hypophysial tube opens behind into the nerve tube. B, later stage. *b.v*, degenerating larval brain-vesicle; *gang.ad*, adult ganglion; *hyp*, hypophysial tube; *n.t*, nerve tube.

Ascidians, grows longer, and acquires an opening to the exterior by fusing with the ectoderm near the mid-dorsal line. This opening is the **anus.** The proximal portion of the intestine swells out to form a globular sac which is termed the **stomach.** From the stomach grows out a pouch, which, by rapid branching, gives rise to a tubular gland called by Willey the **pyloric gland.** This gland, whose branches enswathe the intestine (Fig. 453, C), almost certainly corresponds to the liver of *Amphioxus.*

A median groove, which involves the anus and both atrial openings, is formed in the dorsal surface of the animal. The sides of this groove meet each other, and in this way the **atrial cavity** of the adult is formed. The larval test becomes detached from the ectoderm covering most of the body, which it hereafter loosely enswathes, and the mouth becomes open. The test, however, remains attached to the body near the posterior end of the endostyle. Here a process is formed which projects into the test, and from which fresh test cells are budded off during life.

The **pericardium** arises during free-swimming life. The mode of its development has been satisfactorily made out by Kühn (1893), who has summarized and corrected the accounts of earlier observers. In the free-swimming larva a thickening of the ventral wall of the pharynx can be observed (Fig. 456, A). This thickening is at first a mere doubling of the cells forming the wall of the pharynx in this region. At the close of larval life it becomes a round mass of cells distinct from the pharyngeal wall. In this mass a cavity appears which opens into the pharyngeal cavity (Fig. 456, B). This cavity is, however, soon cut off from the pharynx, and forms the pericardial vesicle. Then its dorsal wall is bent inwards as a median fold. The space between the limbs of this fold is the **cavity of the heart** (Fig. 456, E), and this space communicates with the general body-cavity, which is a blood-space, or haemocoele, formed by the separation of ectoderm and endoderm, and which is a development of the slit-like blastocoele in the embryo. After metamorphosis the pericardial vesicle enlarges and becomes thin-walled, and from its inner walls, which constitute the wall of the heart, muscular fibrils are developed, and then the peristaltic action of the heart begins.

From the pharyngeal wall, in front of and to the sides of the spot from which the pericardium originated, two hollow outgrowths arise and grow backwards. These are the **epicardial tubes** or **epicardia.** They were first described in *Ciona* by Newstead (1894), whose observations were extended and corrected by Damas (1899). According to Damas the left epicardium is larger than the right. Both grow rapidly and form thin-walled vesicles (Fig. 456, F), which constitute the perivisceral cavity of the adult *Ciona*, the primary body-cavity being reduced to a series of blood-sinuses. The conjoined inner walls of the epicardia form a kind of visceral peritoneum, enwrapping heart, pericardium, and intestine. From the left epicardium a plate of cells extends into the process of the body, from which cells are budded into the test. By this plate the cavity of this process is divided into two channels which serve as afferent and efferent blood-streams. The epicardia in compound Ascidians are closely connected with the process of budding; their meaning will be considered later.

When we last considered the pharynx it was provided with two gill-slits on each side, which communicated with the exterior by a lateral ectodermal invagination, the atrial opening. We also described how these two lateral atria become merged in a common median atrial cavity. According to Willey, these primary slits, or **proto-**

stigmata, increase in a remarkable manner at the time of metamorphosis (Fig. 453, C). On each side, each of the slits, at its ventral end, curves inwards towards its fellow of the same side, so that each takes on the form of a U. Then by a downgrowth from the dorsal wall, each U becomes divided into two slits, and so two new slits are intercalated between the first two, which for this reason we numbered No. 1 and No. 4 when we described them above. This process reminds us of the division of the primary slits in *Amphioxus* by the

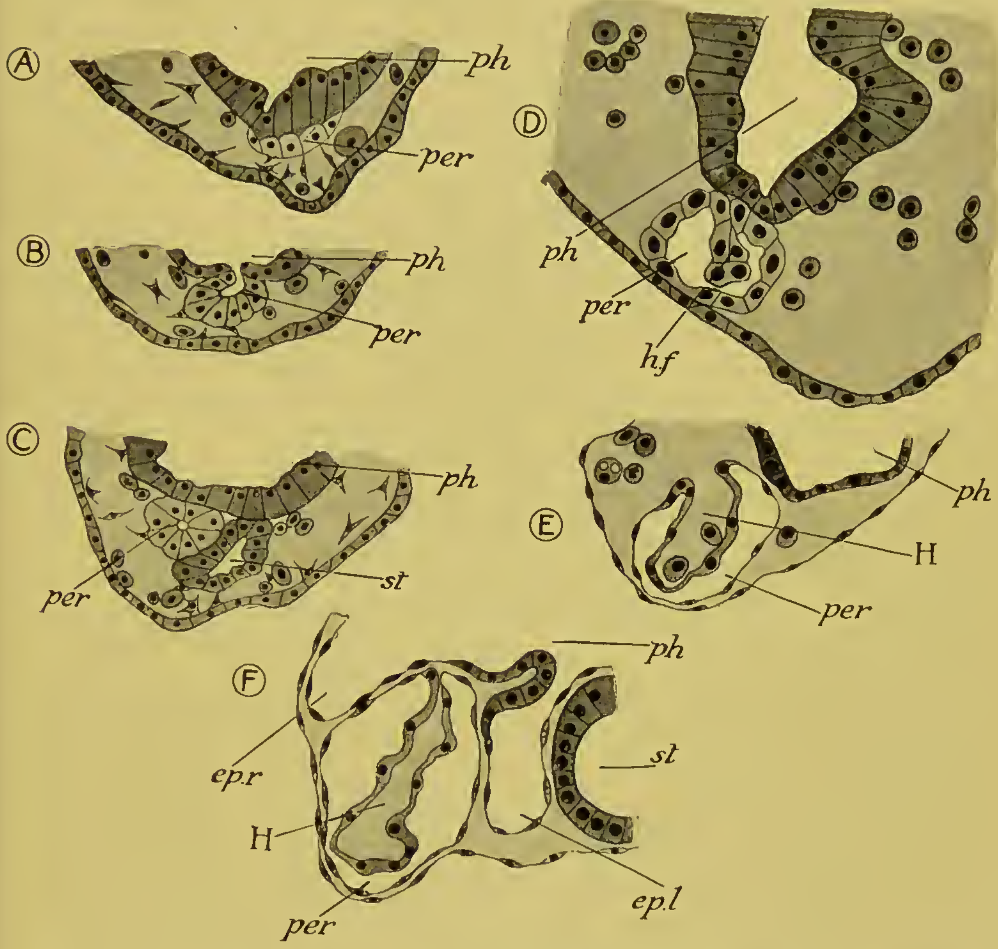

FIG. 456.—Transverse sections through the hinder part of the pharynx of a series of larvae and just-fixed young of the simple Ascidian *Ciona intestinalis*, in order to illustrate the development of the heart pericardium and epicardium. (After Kuhn.)

A, through free-swimming tadpole—the pericardium arises as a thickening of the ventral pharyngeal wall. B, through free-swimming tadpole—a later stage; the pericardium forms a sac opening into the pharynx. C, a still later stage (still free-swimming)—the pericardial vesicles cut off from the pharynx. D, through a just-fixed tadpole—the heart arises as a fold of the dorsal wall of the pericardium. E, through an older specimen than that shown in D—the heart has become hollow and contains blood cells. F, through a still older specimen—the epicardial tubes arise as independent evaginations of the pharynx above the pericardium. *ep.l*, left epicardial tube; *ep.r*, right epicardial tube; *H*, heart; *h.f*, fold which gives rise to the heart; *ph*, pharynx; *per*, pericardium; *st*, stomach.

growth of the tongue-bars. Then another pair of slits arise on each side behind the original second one (No. 4), bringing the total number of protostigmata up to six. It is uncertain whether these new slits (Nos. 5 and 6) arise as independent perforations of the pharyngeal wall or by the bisection of a single U-shaped slit on each side, but the latter hypothesis seems to be the more probable.

Since the long axis of the pharynx in the larva becomes its vertical axis in the adult, it is clear that these six protostigmata must form a vertical row; and, when they become subdivided, they give rise to six transverse rows of **stigmata.** A stage with six undivided protostigmata on each side has been found in many simple Ascidians. The subdivision of the protostigmata has been described by De Selys-Longchamps (1901), whose account has been confirmed by Fechner (1907). According to him, bud-like outgrowths are formed on the bars separating Nos. 1 and 2, 3 and 4, 5 and 6, respectively, *i.e.* the tongue-bars. The outgrowths eventually unite and form the internal longitudinal bars of the adult pharynx.

The subsequent multiplication of the stigmata of these six primary transverse rows has been investigated by De Selys-Longchamps (1901), and by Damas (1901) and Fechner (1907). It occurs by the transformation of all of them into U's, like the first-formed U's; these have their convexities towards the endostyle and their limbs pointing towards the mid-dorsal line of the pharynx. Then these U's become divided by dorsal downgrowths, and in this way the number of transverse rows of stigmata is doubled. The number of slits in a transverse row is increased by the cutting off of small portions of each slit from the rest by outgrowths from the edges. The five large broad bars containing blood-vessels, which separate the six primary slits, are recognizable in the adult stage of those individuals which have been produced, not by budding, but by the metamorphoses of larvae.

The origin of the **genital organs** has not been clearly seen in *Ciona*, but has been described in *Molgula*, another genus of simple Ascidians, by De Selys-Longchamps and Damas (1902). In this genus, in which a remnant of the cerebral vesicle containing the otolith persists throughout life, one is able to see in the young fixed form, when it has only two gill-slits, a small rounded mass of mesoderm cells on the ventral side of the pharynx beneath the bar separating the two gill-slits (Fig. 457). On the left side a similar mass is seen in the concavity of the last curve of the intestine. As these lobes grow they become divided into a superior mass, the rudiment of the **ovary,** and a lower one, which is the beginning of the **testis.** The ovary is hollow; the upper wall of the cavity is flat, the lower consists of the germ cells. Both rudiments grow in length and consist of bands whose length is transverse to the length of the pharynx. The rudiment of the testis becomes covered with rounded buds (Fig. 457, C), which finally constitute the ampullae where the spermatozoa are produced; ultimately the rest of the gland becomes hollowed out to form the **vas deferens.** The **oviduct** is also formed from the upper, sterile portion of the rudiment of the ovary. In this genus there is, therefore, a right and a left ovary, and a right and a left testis. It must be remembered that in *Ciona* there is only one ovary and one testis.

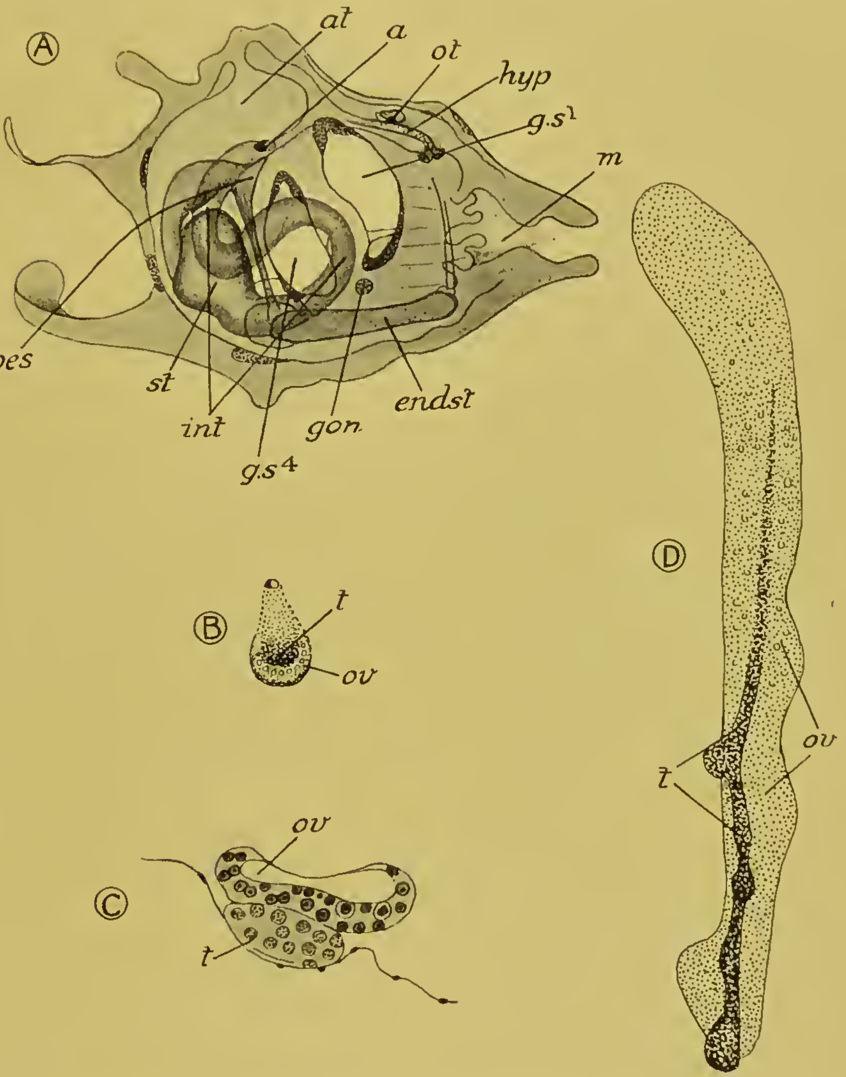

FIG. 457.—Illustrating the development of the genital organs in the simple Ascidian *Molgula ampulloides*. (After De Selys-Longchamps and Damas.)

A, young Ascidian seen from the side showing first trace of the genital organs. B, the genital rudiment of an older specimen showing incipient differentiation into ovary testes. C, transverse section through a genital rudiment of a still older specimen showing the hollow ovary and the solid testes. D, a considerably older genital rudiment showing the formation of ampullae by the testes. *a*, anus; *at*, atrial cavity; *endst*, endostyle; *gon*, rudiment of genital organ; gs^1, gs^4, the first two gill-slits; *hyp*, hypophysial canal; *int*, intestine; *m*, mouth; *oes*, oesophagus; *ot*, otolith; *ov*, ovary; *st*, stomach; *t*, testes.

EXPERIMENTAL EMBRYOLOGY OF SIMPLE ASCIDIANS

We must now describe some illuminating experiments which Conklin (1905, 1906) performed on the egg of *Cynthia*. The course of the ordinary development of this form strongly suggests the view that the different coloured cytoplasms, found in the unsegmented egg, are definite organ-forming substances; but, of course, this is not necessarily so, and attempts to correlate coloured substances in the eggs of the sea-urchin, *Strongylocentrotus*, with organ-forming substances have resulted in failure.

Conklin found that the ordinary means of isolating the blastomeres of segmenting eggs were not applicable to *Cynthia*. If eggs were deprived of their membranes by shaking they perished; and the same result followed if an attempt were made to cut them into two by means of a sharp scalpel. If, however, the segmenting eggs were "spurted," *i.e.* were sucked violently up in a pipette and blown out with equal violence a considerable number of times, then, frequently, part of the egg, *i.e.* one or more blastomeres were killed, but the survivors continued their development and in some cases larvae emerged.

If one of the first two blastomeres be killed in this way the survivor segments exactly as if its dead sister were still present. In this way half-gastrulae are formed, which are like normal gastrulae laid open lengthwise; the edge of the cup is bordered by a quarter circle of notochordal cells in front, and by a quarter circle of mesenchyme and muscle cells behind. When these gastrulae become larvae the missing parts are not regenerated. There are muscle cells and mesenchyme cells only on one side of the body, and though the ectoderm cells extend round so as to cover in what would be the naked side of the notochord, yet the notochord itself is only formed from one row of cells, not two as in the normal larva (Fig. 458, B). At the posterior extremity of the tail the muscles of the injured side do, to a limited extent, grow on to the uninjured side.

If the egg is "spurted" in the 4-cell stage, any one of the four blastomeres may be killed, or the two anterior or the two posterior; or only one blastomere may remain alive, and this latter may be any of the four. In what we may call anterior half-larvae, neural plate and notochordal cells are formed, but there is no trace of muscle cells or of mesenchyme; the neural plate is never enrolled so as to form a nerve tube, the notochordal cells form only a plate, and invagination does not take place. In posterior half-larvae, muscle and mesenchyme cells are well formed, but there is no trace of neural plate or notochord, and the endoderm forms a solid mass; and, since there is no notochord, the muscle cells meet in the mid-dorsal line. The mesenchyme cells form two masses beneath the muscle cells, and are separated by two rows of endoderm cells. The embryo remains rounded and shows no trace of the tadpole form. Quarter embryos are still more imperfect, but what develops out of them corresponds exactly to what would have developed out of them had their sister blastomeres remained alive.

Conklin, therefore, may fairly claim to have proved that these coloured cytoplasms are definite organ-forming substances. He has proved that similar substances exist in the eggs of *Ciona, Molgula,* and *Phallusia* (1911), only that these substances are not distinguishable from one another in the living egg, but require staining to differentiate them from one another.

But now the question arises: if the organs of the larva are determined by substances situated in definite regions of the unsegmented egg, whence do those substances come? The answer to this is, that one of them, the clear ectoderm-forming substance, is actually contained in the nucleus. Further, Schaxel (1910) has shown that, in the developing oocyte, chromatin is emitted from the nucleus, and that the slaty-blue yolk spherules grow at the expense of these chromatin particles. Hence we may boldly assert that the cytoplasm of the ripe egg is differentiated by the egg-nucleus.

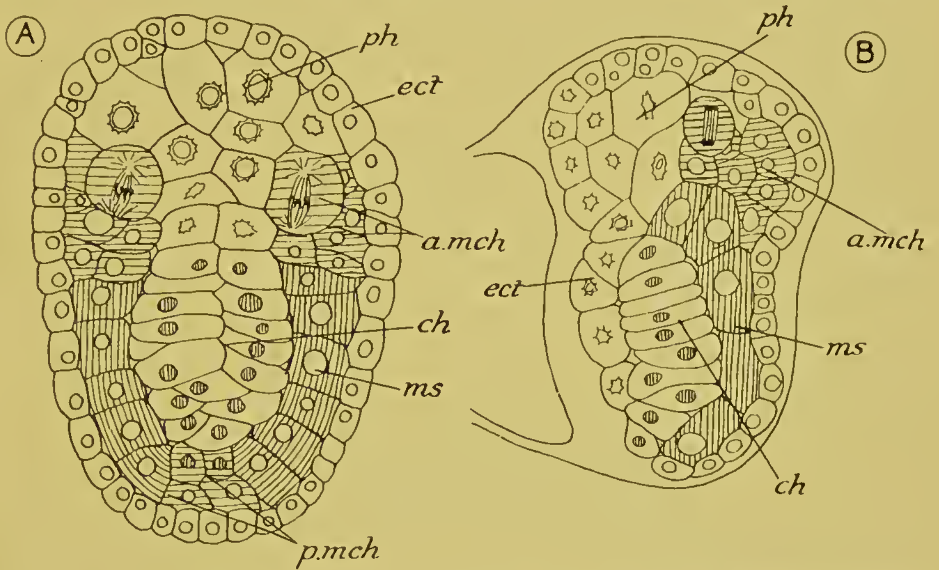

FIG. 458.—Illustrating the result of the development of single blastomeres of the 2-cell stage of the egg of *Cynthia partita*. (After Conklin.)

A, frontal longitudinal section of young tadpole of *Cynthia partita* developed from normal egg. B, frontal longitudinal section of young tadpole of *Cynthia partita* developed from 2-cell stage in which the left blastomere was killed. *a.mch*, anterior mesenchyme (corresponding to the superior mesenchyme of Fig. 446); *ch*, notochord; *ect*, ectoderm; *ms*, mesodermal band; *ph*, pharynx; *p.mch*, posterior mesenchyme (corresponding to the inferior mesenchyme of Fig. 446).

But although the substances are *formed* under the influence of the egg-nucleus, they are *arranged* under the influence of the spermatozoon. As we have seen, the entering spermatozoon drags the yellow substance after it, and thence determines the position of the muscle-forming material. If two spermatozoa enter the egg, then the yellow substance forms two aggregations instead of one. In the egg of *Cynthia partita* Nature has provided us with an ocular demonstration of the existence of organ-forming substances.

OTHER GROUPS OF ASCIDIANS

We must now take a brief glance at the modifications which the life-history which we have described undergoes in other groups of Ascidians. As already indicated, the other simple Ascidians, so far as they have been examined, have a development which is, cell for cell, identical with that of *Cynthia*. To this statement, however, certain species (but not all) of the genus *Molgula* form an exception.

In *Molgula ampulloides*, as described by Damas (1902), the early development is identical with that of *Cynthia*, but the tail rudiment remains short, and the hinder part of the nerve tube, the notochord, and the tail muscles, undergo degeneration before the larva escapes from its membrane. The notochord and muscles are indeed converted into a mass of fatty cells which forms a rounded protuberance at one side of the larva. The larva cannot swim, and tumbles at once to the bottom, where it attaches itself by long finger-like processes of the test. The atrial cavity is formed by a single mid-dorsal involution of the ectoderm, which forks below into right and left pouches; each of these pouches comes into contact with two evaginations of the pharyngeal wall which are the endodermal portions of the first two gill-slits on either side.

In the Ascidiae compositae, the larva differs from that of simple Ascidians in the precocious appearance of adult features; thus the atrial cavity generally attains the form of a single dorsal cavity with median opening, whilst the larva is still free-swimming. The chin region of the larva is already disproportionately large, and the mouth and atrial openings are consequently close together. In some cases, as for instance in *Clavelina*, the eggs are small; but in other cases, such as *Distaplia*, the eggs are larger and more yolky, and the endoderm is in the form of a solid mass of cells like those at the lower pole of the frog's egg. In such cases the archenteric cavity is formed by a separation of these cells from one another, not by an invagination, and there is never an open blastopore. The neuropore never closes, but persists until it becomes converted into the opening of the sub-neural gland.

In Ascidiae luciae, represented by the single genus *Pyrosoma*, the eggs are still larger and each individual produces only one egg. The yolk is so massive that it remains largely unsegmented, and its development is, like that of Cephalopoda, of the meroblastic type, in which a germ disc or **blastoderm** rests on an unsegmented mass of yolk. The test cells, or follicle cells, wander inwards amongst the blastomeres during the progress of segmentation (Fig. 459). Many of them die and degenerate and are used as food, but according to Julin (1912), who has given the latest and most satisfactory account of the development of this form, many of the test cells survive and give rise to the **phosphorescent organs** of the adult (*phos*, Fig. 460). In the yolk there are nuclei which are derived by division from the nuclei of the blastomeres, and which we may term **yolk cells.** They are surrounded by islands of cytoplasm (*y.c*, Fig. 460).

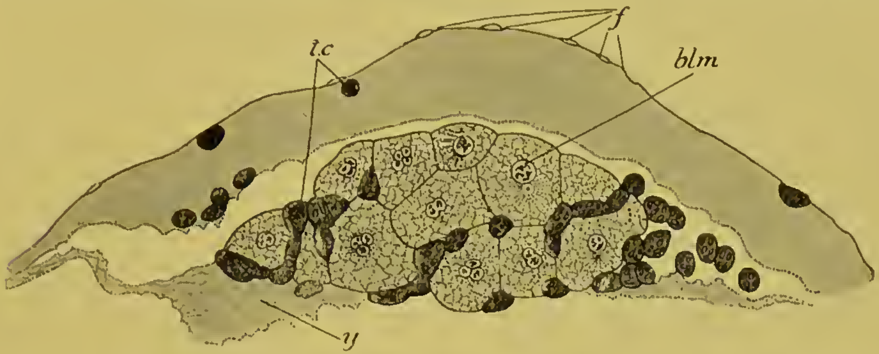

Fig. 459.—Section through the segmenting of *Pyrosoma giganteum* at the stage of thirty-six blastomeres. (After Julin.)

blm, blastomere; *f*, follicle cells; *t.c*, test cells—some have wandered in amongst the blastomeres; *y*, unsegmented yolk.

Between the blastoderm and the yolk a cavity appears which is the archenteric cavity, and at this stage, according to Julin, the blastoderm consists, throughout the greater part of its extent, of two layers. The outer of these layers constitutes the ectoderm, and the inner, which forms the immediate roof of the archenteric cavity, is endoderm. The two layers pass into each other along a definite lip which Julin identifies with the dorsal and anterior lip of the blastopore, corresponding to the region *x* in the gastrula of *Amphioxus.*

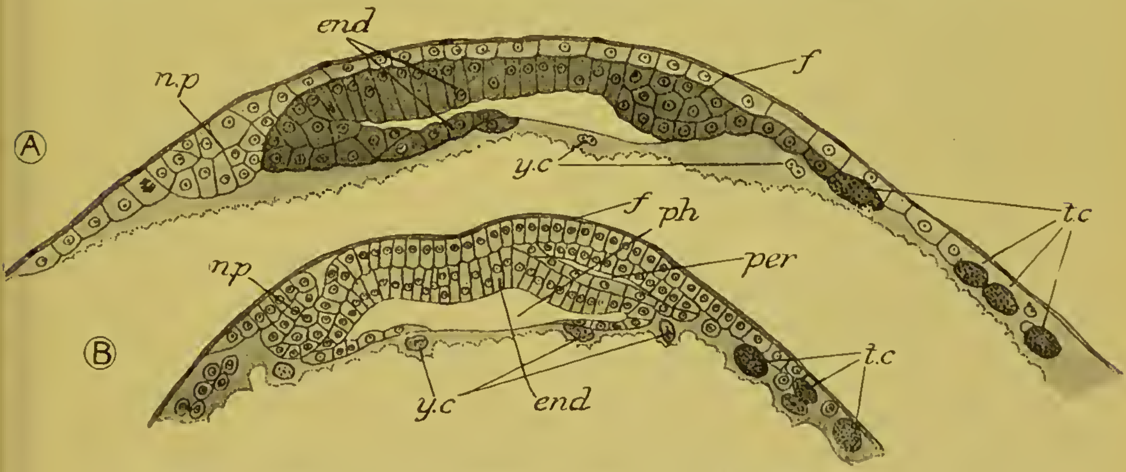

Fig. 460.—Longitudinal sections through two embryos of *Pyrosoma giganteum*, in order to show the formation of organs in the Cyathozooid. (After Julin.)

A, section through younger embryo. B, section through older embryo. *end*, endoderm forming roof of archenteron and beginning to extend on to its floor; *f*, follicle with nuclei; *n.p*, nerve plate formed by an ectodermic thickening which becomes invaginated; *per*, pericardial tube; *ph*, pharynx; *t.c*, test cells; *y.c*, yolk cells.

In later stages of development the archenteric cavity acquires a floor of cells, produced mainly by the increase in number and adhesion of the yolk cells, which represent the white cells at the lower pole of the frog's egg. Meantime the ectoderm, extending out from the anterior edge, *i.e.* the opposite side of the blastoderm to that of the region *x*, grows as a simple sheet of cells over the yolk, and so eventually the blastopore is closed. The archenteron becomes the **pharynx,** no tail endoderm being formed.

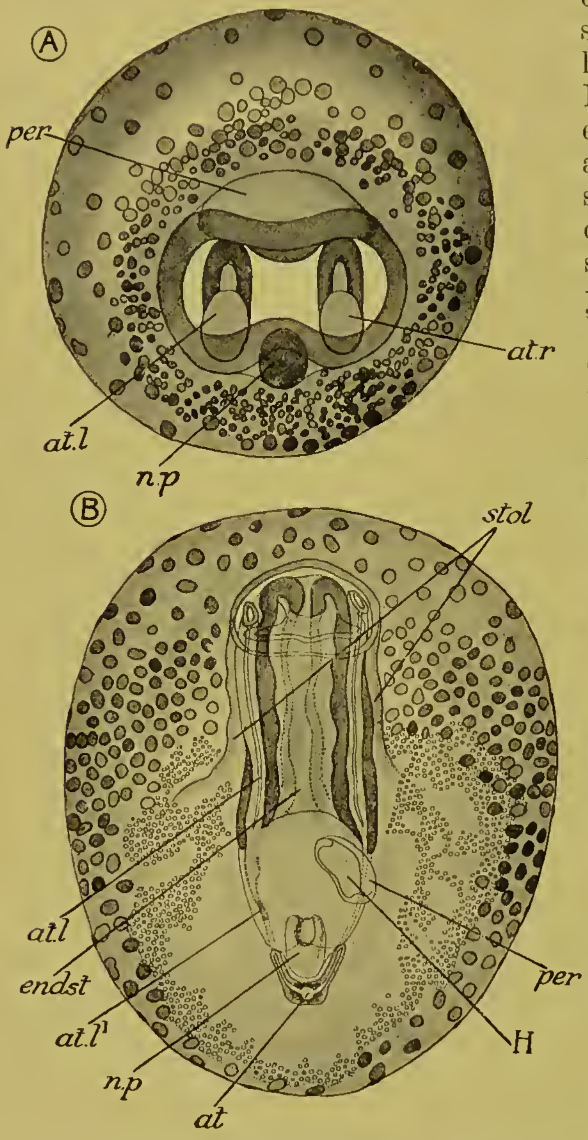

FIG. 461.—Two embryos of *Pyrosoma giganteum*, viewed from the dorsal surface in order to show the formation of organs in the Cyathozooid. (After Julin.)

A, younger embryo. B, older embryo which has already formed a stolon. *at*, common atrium formed by the junction of the two atrial tubes; *at.l*, left atrial tube; *at.l*^{1}, obliterated portion of the left atrial tube; *at.r*, right atrial tube; *endst*, endostyle; *H*, heart; *n.p*, nerve-plate; *per*, pericardial sac; *stol*, stolon.

The formation of organs is now begun. The **nervous system** arises as a median dorsal invagination of ectoderm in front of the region *x*. Behind it a median pit arises, which is the rudiment of the **cloaca** or median atrial opening. On the sides of the pharynx two ectodermal grooves are formed which are the **atrial invaginations.** They become largely separated from the exterior so as to form tubes with narrow openings directed dorsally. As growth goes on their openings are forced farther and farther upwards, and become involved in the median invagination, alluded to above, which forms the cloaca. Each **atrial tube** communicates with the pharynx, on its inner side, by a narrow slit which is a rudimentary **gill-slit.**

The **pericardium** arises as two ventral outgrowths of the pharynx, which, however, speedily fuse into one and constitute a single vesicle. The single vesicle sends out two prolongations, a right and a left; of these the left becomes solid and atrophies, but the right swells and forms the persisting **heart** and pericardium of the embryo, whilst the rest of the vesicle disappears.

On the floor of the pharynx the **endostyle** is formed as a large median groove with thick prominent lips, and behind this there grows out what Julin calls a prolongation of the pharynx, but which we (following Hjört) regard as two enormous epicardial tubes fused together. This prolongation curls itself round the yolk, and from it, in a manner to be described shortly, the first buds arise. It remains to be added that, when the atrial involutions have become united with the median cloacal opening, the two rudimentary gill-slits disappear and are replaced by a single dorsal median opening into the pharynx.

If this description has been followed, it will be seen that the first individual to be formed by the development of the fertilized egg in *Pyrosoma* is of a very degenerate character. It possesses no trace of a mouth or of an intestine, and no trace of notochord or caudal muscles. In the neighbourhood of the blastoporal rim a ring of mesenchyme is produced. This vestigial individual is called the Cyathozooid, in order to distinguish it from the more perfect individuals produced by budding.

When we pass to the Thaliaceae we find, in the genus *Salpa*, a form in which the egg develops directly into the adult form without the intervention of a tailed larval stage. In this case the egg remains in the oviduct in the mother, only moving down from the ovary to a brood-chamber at the mouth of the oviduct; whereas in *Pyrosoma* the egg is spawned into the sea. The egg, after dividing into a few blastomeres, is invaded by follicle cells which wander in between the blastomeres and surround them. According to Salensky (1883), the blastomeres perish and the embryo is built up out of follicle cells.

In view of the fact that Salensky (1891) has made somewhat similar statements about *Pyrosoma*, in which he alleges that test cells become blastomeres, and that Julin has shown this view to be erroneous, it may be doubted whether Salensky's statements about *Salpa* are any more reliable. Moreover, in the case of *Salpa*, it is very difficult to distinguish between blastomeres and follicle cells. It seems more probable that it is the follicle cells which perish and are eaten by the separated blastomeres, which then reunite. Similar cases of the separation and subsequent reunion of blastomeres are known amongst Platyhelminthes.

The solid mass of cells constituting the embryo sits, rider-like, on a knob of maternal tissue, inside which there is a great development of blood-vessels. This knob constitutes a nutritive organ or **placenta**. The blood of the mother seems to diffuse through to the tissues of the embryo. An ectoderm is differentiated from the peripheral layer of the embryonic mass, and the placental knob is overgrown by a flap of this layer. From a thickening of the ectoderm the nervous system is developed.

Two lateral portions of the inner or endodermic mass become distinguishable from the rest, by their histological character, as

mesoderm. A posterior mass is the rudiment of the **elaeoblast,** a protuberant mass of fatty globules which probably represents the last vestige of the lost larval tail. The pharyngeal cavity then appears in the centre of the median portion of the endodermic mass. It is nearly divided into two by a median dorsal infolding, the rudiment of the adult **"gill."** The cloacal cavity appears as a median invagination of ectoderm, and unites with the pharynx at the sides of this so-called "gill."

The nervous system separates from the ectoderm and becomes hollowed out, showing a transient indication of division into three chambers, which have been compared to the three brain-vesicles of the embryos of higher Vertebrates (*n.t*, Fig. 464). The neural tube becomes connected with the pharynx close to where the mouth is formed as an invagination. The mesodermic mass on each side gives rise to the characteristic hoop-like muscles which are the adult organs of locomotion. The elaeoblast is now gradually absorbed, the embryo wrenches itself loose, breaking off the placental knob and carrying away with it this portion of the maternal tissues embedded in its body, where it is gradually absorbed, though its remains can be recognized for a long time.

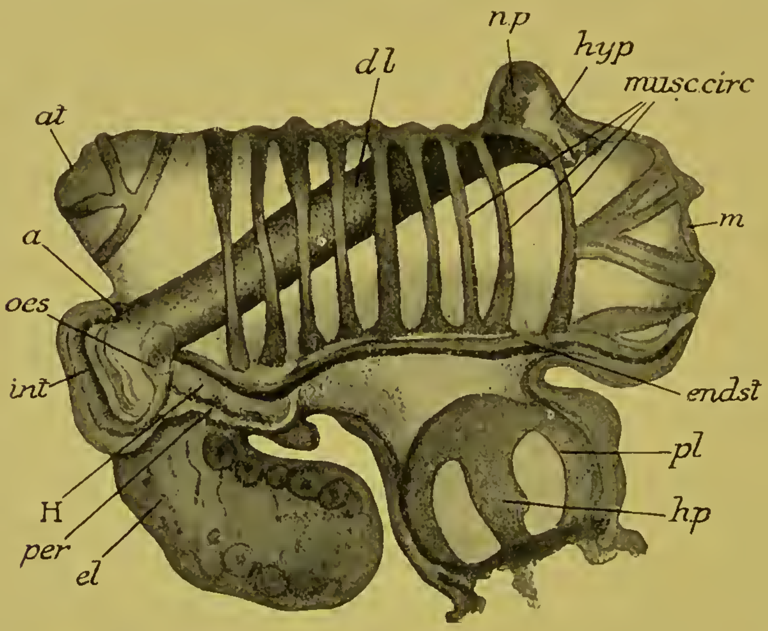

FIG. 462.—The embryo of *Salpa fusiformis* attached to the maternal placenta, seen from the side. (After Salensky.)

a, anus ; *at*, atrial opening ; *d.l*, dorsal lamina (remnant of dorsal wall of pharynx) ; *el*, elaeoblast ; *endst*, endostyle ; *H*, heart ; *h.p*, blood-forming organ in the placenta ; *hyp*, hypophysial canal ; *int*, intestine ; *m*, mouth ; *musc.circ*, circular bands of muscle ; *n.p*, nerve ganglion ; *oes*, oesophagus ; *per*, pericardium ; *pl*, placenta.

In *Doliolum*, the other well-known genus of Thaliaceae, the egg is shed into the sea, and its early development seems to resemble that of simple Ascidians; but the tailed larva shows some quite peculiar features. The pharynx remains quite small, and there is an enormous ectodermic vesicle at the root of the tail which obviously serves for flotation. In front of this there is a mesodermic mass, and behind it are situated two mesodermic masses, and behind these again the notochord with its longitudinal muscles.

The nervous system is represented by a very large solid mass of cells—a brain-vesicle being apparently absent. The front mesodermic masses give rise to the hoop-like muscles of the pharynx; the atrial cavity arises as a median invagination, forking below, and each fork communicates with the pharynx by a series of undivided "protostigmata." As the hoop-like muscles develop the great ectodermic vesicle shrinks, and finally the embryo differs from the adult only in carrying tail and notochord. These are finally absorbed and the development is then complete.

The Ascidiae luciae and the Thaliaceae agree in being purely pelagic forms which pass the whole of their lives swimming in the open sea. The tailed larva cannot, therefore, fix itself to any solid substratum. To meet these altered conditions the development undergoes three different modifications in *Pyrosoma*, *Salpa*, and *Doliolum* respectively.

In *Pyrosoma* the embryo is a mere sketch of the typical larval form; it begins to bud before it is half-developed, and founds the colony round the remains of the yolky egg, after which the original person disappears. In *Salpa* the egg must have originally developed into a tailed larva, but this larva fixed itself to the maternal atrial wall at the mouth of the oviduct, and there grew till it was ready to break loose. Finally, in *Doliolum*, the larval organs, tail, and notochord are *retained* till the adult form has been reached.

ASEXUAL REPRODUCTION IN UROCHORDA

The asexual reproduction of the Urochorda is a most fascinating subject, and has attracted an immense amount of research which is embodied in an extensive literature. The whole subject, however, has been reviewed in a most masterly way by Hjört (1896), whose views have stood unchallenged for the last eighteen years. Ritter (1897), the only worker who has since then published an important paper on the subject, confirms Hjört in all important matters and endorses his views. It would be waste of time, therefore, to trouble the reader with the views of earlier workers whose ideas on the subject were by no means clear. Pizon, however, both before (1891) and since Hjört's paper appeared (1905), has made a special study of the extraordinary condition of affairs prevailing in the family of compound Ascidians termed Diplosomidae.

According to Hjört, then, there are two main types of bud-formation found amongst Urochorda, which, so far as we can see, must have been independently evolved. These are **pallial budding,** found in *Botryllus* and its allies; and **stolonial budding**, found amongst all other Tunicata.

Stolonial budding has been thoroughly studied in the genus *Clavelina* by Van Beneden and Julin (1887), and the results of these authors have been completely confirmed by Hjört's studies on *Perophora* and *Distaplia*. In all three genera the two epicardial cavities grow out from the pharynx, even in the free-swimming larva, and as soon as fixation takes place they become long and fuse at their distal ends into a single cavity. Van Beneden and Julin imagined that the pericardium was cut off from this cavity, but that, as we have seen, is a mistake: the pericardium originates earlier, as a median evagination of the pharynx. The conjoined epicardial cavities collapse, and their walls then form a thin plate of cells, which projects into a ventral protrusion of the ectoderm which forms the **stolon.** It forms a median septum in this stolon, which divides the afferent and efferent blood-streams from each other; but as this septum does not reach quite to the tip, the streams pass into each other here.

New buds are formed as lateral branches of the stolon. Branches of the epicardial septum are continued into these branches, and these pieces of the septum develop into sacs by the reappearance of the lost cavity of the epicardial tube (Fig. 463). The young bud is therefore formed from a **double-walled vesicle,** of which the outer layer is furnished by the parental ectoderm, whilst the inner layer arises from the epicardial tube. Between these two layers are contained a certain number of loosely aggregated mesoderm cells.

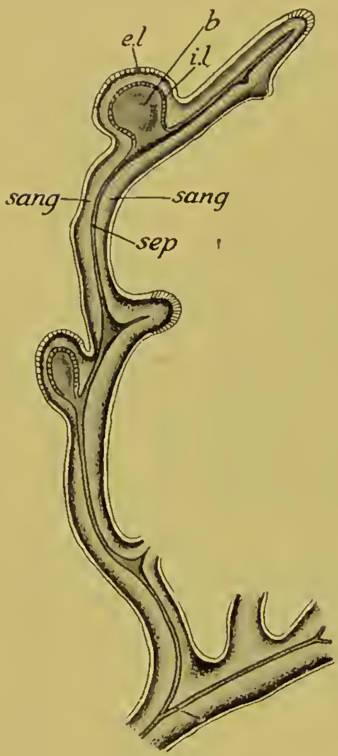

FIG. 463.—Budding stolon of *Perophora listeri* showing the development of buds from the septum of the stolon. (After Hjört.)

b, blood; *e.l*, external layer of bud rudiment; *i.l*, internal layer of bud rudiment; *sang*, blood-space; *sep*, septum of the stolon, a double layer of cells.

As Hjört shows, the building up of the organs of the young **"blastozooid,"** as the "person" produced by budding is termed, takes place in a very different manner from that in which it occurs in the embryo produced from the fertilized egg. Thus the atrial cavity arises by the downgrowth of two septa, which divide the inner vesicle into a median and two lateral portions; the median is the rudiment of the pharynx, the lateral of the atrial cavities. *The nervous system arises as a dorsal hollow outgrowth of the inner vesicle, at the border of the left and median divisions*; it grows forwards and acquires a new opening into the vesicle at the front end. This new opening becomes the **sub-neural gland**, or hypophysial tube; the other opening disappears. No protostigmata are formed; the definitive stigmata arise as small perforations of the pharyngeal wall. The intestine arises as an outgrowth from the hinder wall of the pharynx; the pericardium as a median evagination of the ventral wall of the same organ. The muscles and the genital organs arise from the mesodermal cells (Fig. 464).

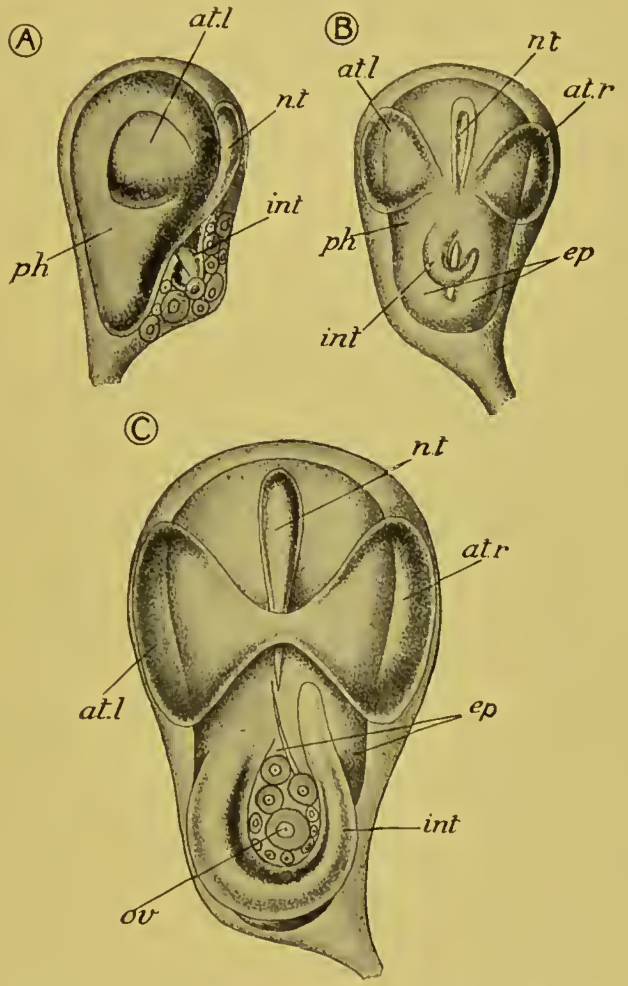

FIG. 464.—Stages in the development of the bud of *Distaplia magnilarva*. (After Hjört.)

A, lateral view of young bud in which the two atrial rudiments are still separated. B, dorsal view of young bud in which the two atrial rudiments are still separated. C, dorsal view of older bud in which the atrial rudiments have united in the mid-dorsal line. *at.l*, left atrial rudiment; *at.r*, right atrial rudiment; *ep*, epicardial tubes; *int*, rudiment of intestine; *n.t*, nerve tube—rudiment of adult ganglion; *ov*, ovary; *ph*, pharynx.

In *Pyrosoma*, *Salpa*, and *Doliolum*, the stolon arises in just the same manner as in *Clavelina*. In *Pyrosoma*, as we have already learnt, the rudimentary embryo is called the **Cyathozooid**; the stolon gives rise to four "blastozooids" known as "**Ascidiozooids,**" which form a cycle according to Julin (1912). The ectodermal peribranchial or atrial tubes of the Cyathozooid extend backwards into the Ascidiozooids, and give rise to the atrial cavities of these persons by segmenting off four pieces; but this, according to Hjört, does not hold for buds subsequently produced. The atrial cavities of the later buds arise from solid proliferations of the walls of the inner vesicle, which subsequently become hollowed out, and obviously correspond to the lateral compartments of the inner vesicle of the bud of *Distaplia*. The nervous system arises as a pair of outgrowths from these two lateral vesicles, which grow inward and unite in a mid-dorsal tube, which then forms a new connection with the central vesicle in front.

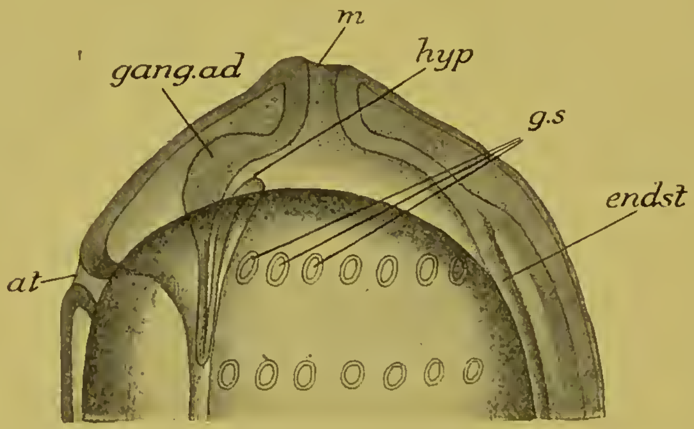

FIG. 465.—Lateral view of old bud of *Distaplia magnilarva*. (After Hjört.)

at, opening of single atrial cavity; *endst*, endostyle; *gang.ad*, adult ganglion, a thickening of the dorsal wall of the hypophysial tube; *g.s*, first gill-slits appearing as perforations of the pharyngeal wall; *hyp*, hypophysial tube; *m*, mouth.

The posterior connections become solid and form a transverse nerve (Fig. 466).

In *Salpa*, the buds formed by the stolon remain connected with one another for a considerable time, and constitute in this way a train of "blastozooids," which is dragged along by the **oozooid**, as the person produced from the egg is termed, and which thus acts as engine to the train. As the blastozooids attain a respectable size they drop off and lead independent lives; they constitute the sexual generation.

In *Doliolum* the buds break loose from the stolon whilst they are in a most immature condition, and climb by amoeboid movements over the surface of the parent, till they reach a posterior horn of the test situated in the mid-dorsal line. To this horn they attach themselves in three longitudinal lines. Those in the lateral lines become **trophozooids,** without muscles and with wide mouths, which serve to nourish the colony; those in the median line become like the parent, except that they have a different number of muscular bands. It is uncertain whether these median buds, termed **phorozooids**, break loose and develop into individuals with sexual organs, or whether they produce ventral stolons from which the true sexual generation arise as buds.

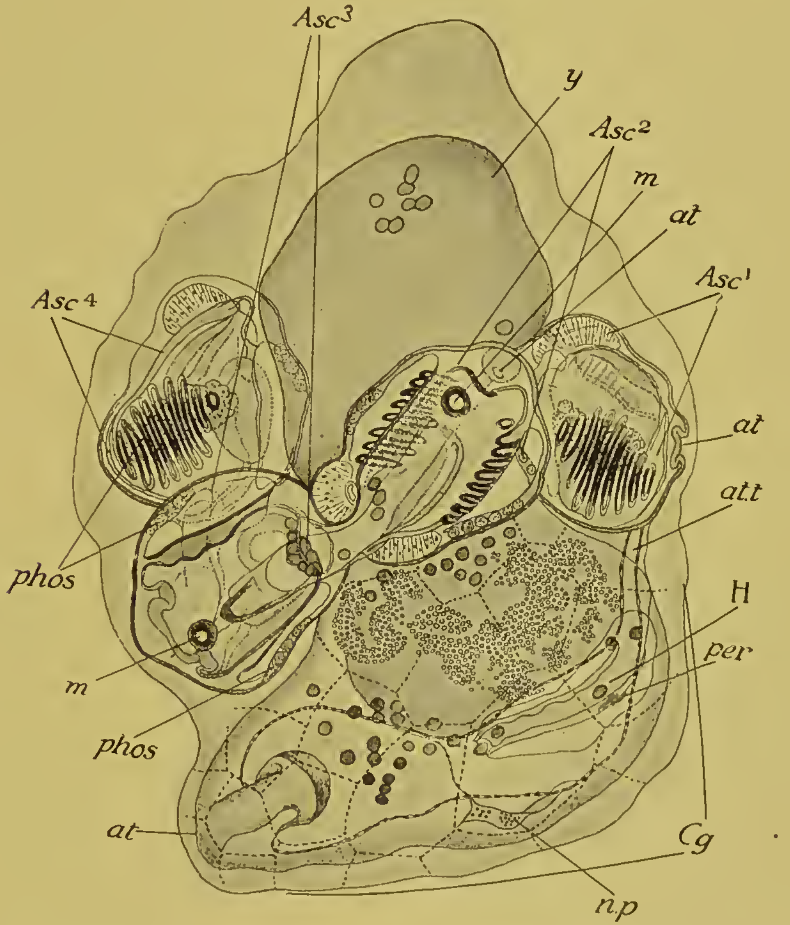

FIG. 466.—Primordial colony of *Pyrosoma giganteum* consisting of four Ascidiozooids coiled round the degeneration Cyathozooid. (After Julin.)

*Asc*1-*Asc*4, the four Ascidiozooids; *at*, common atrial openings of an Ascidiozooid and also of the Cyathozooid; *at.t*, persistent atrial tube; *Cg*, Cyathozooid; *H*, heart of Cyathozooid; *m*, mouth of Ascidiozooid; *np*, nerve-plate of Ascidiozooid; *per*, pericardial sac of Ascidiozooid; *phos*, phosphorescent organ consisting of an agglomeration of test-cells; *y*, unsegmented yolk of Cyathozooids.

The buds of *Doliolum* are in so far peculiar that the inner vesicle is represented by a solid mass of cells, in which strings of cells are gradually differentiated which develop into pharynx, atrial cavities, muscular tissues, and nervous system.

In *Botryllus* and its allies the buds arise as two layered vesicles, and their development is exactly what has been described in the case of *Distaplia*. But whilst the outer wall of the vesicle arises as an evagination of the parent ectoderm, *the inner arises as an evagination of the parent atrial cavity* (Fig. 467). *No epicardia exist in the Botryllidae.* The young "oozooid" produced from the egg perishes after having given rise to the first buds, which then give rise to the rest of the colony (Fig. 467).

Finally, in the family Diplosomidae we have a most extraordinary condition of affairs, which has been elucidated by the researches of Pizon (1905). Budding begins by the development of epicardia in the embryo, before the larva escapes from the egg-membrane. During the larval life a perfect blastozooid is produced, so that the tailed larva possesses *two branchial sacs, two intestines, and two nervous systems, but only one notochord.* One of the nervous systems, *i.e.* of the blastozooid, is devoid of a sense-vesicle. When the larva fixes itself budding continues in both blastozooid and oozooid. The epicardial stolon, however, produces only a pharynx, nervous system, and the rudiment of an oesophagus. *This oesophagus, instead of developing a new gut, joins the oesophagus of the parent.* At the same time an independent bud is produced as an evagination of the rectum (Fig. 468, B), and forms a new rectum though still retaining its connection with the old one.

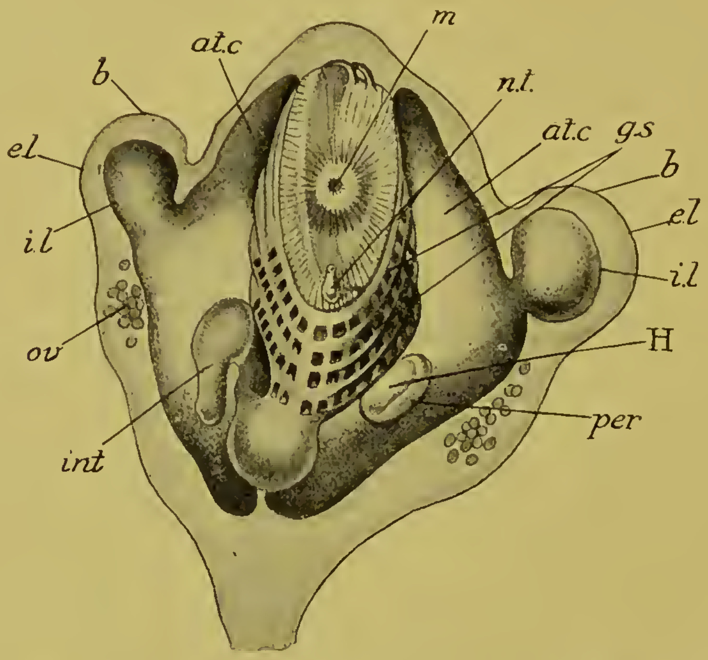

FIG. 467.—Bud of a Botryllid almost completely developed and bearing two young buds; seen from the dorsal side. (After Hjört.)

at.c, atrial cavity; *b*, young bud rudiment; *e.l*, external layer of young bud rudiment; *g.s*, gill-slits; *H*, heart; *i.l*, internal layer of young bud rudiment—this is seen to be a prolongation of the lining of the atrial cavity of the mother; *int*, intestine; *m*, mouth; *n.t*, nerve tube (including hypophysial tube); *ov*, ovary; *per*, pericardium.

Then one of two things may happen. Either a new stomach and intestine are developed from a third bud, which arises from the old oesophagus just where the epicardial bud joins it (Fig. 468, D); and

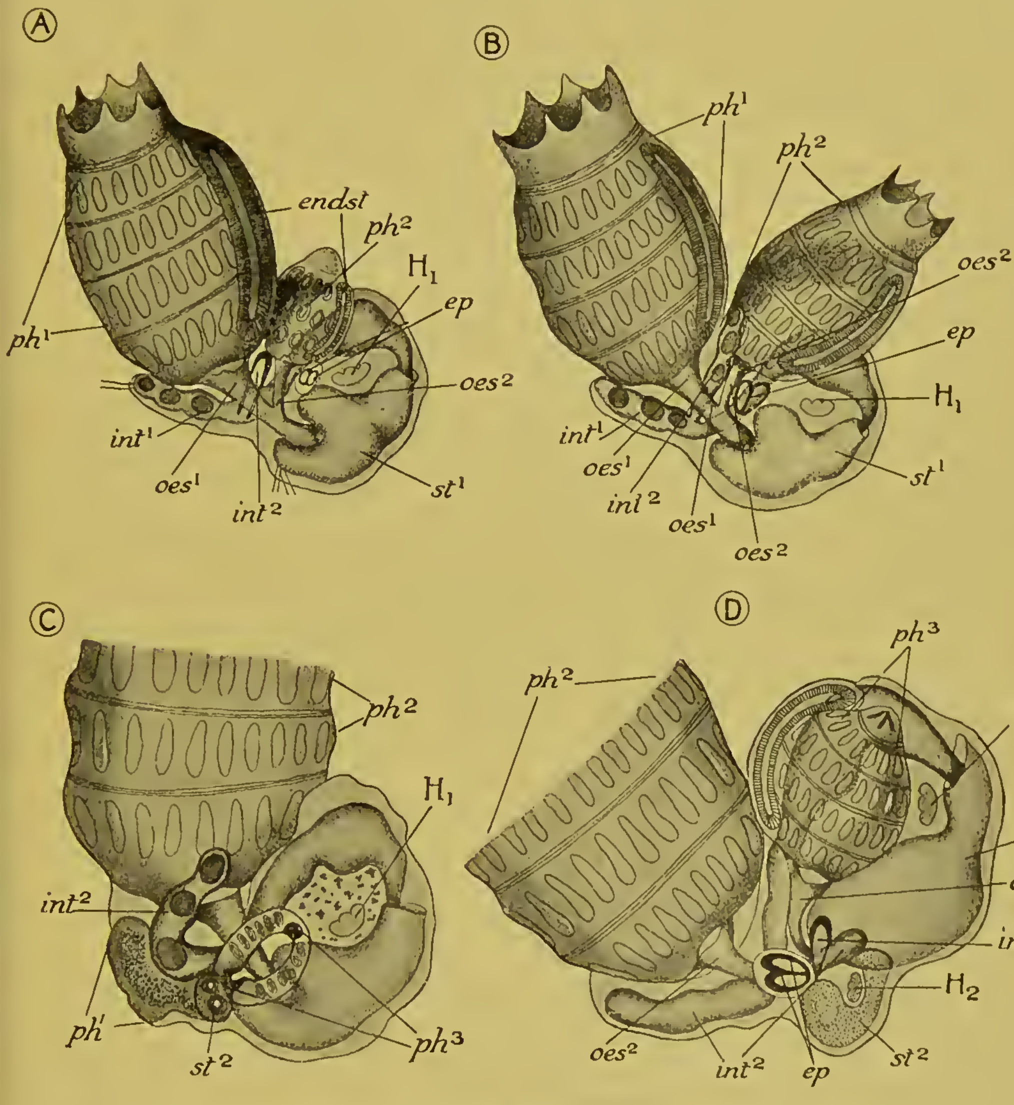

FIG. 468.—Four stages in the budding of a Diplosomid Ascidian. (After Pizon.)

A, the oozooid (*i.e.* the organism produced by the metamorphosis of the larva) has produced by separate buds a new pharynx and oesophagus and a new intestine. B, the new organs have greatly increased in size. C, the pharynx of the oozooid is reduced to a degenerate mass its oesophagus and intestine have disappeared, but the second pharynx has produced by budding a third pharynx; a second stomachic loop has been produced. D, the third pharynx has increased in size and has developed an oesophagus and a third intestine has been produced. A second heart has been produced from the second pharynx, and the second stomachic loop has become functional. *endst*, endostyle; *ep*, epicardial outgrowths from second pharynx; H_1, H_2, the hearts; int^1, int^2, int^3, the intestinal rudiments; oes^1, oes^2 oes^3, the oesophageal rudiments; ph^1, ph^2, ph^3, the three pharynges; st^1, st^2, the two stomachs.

so a compound organism arises with two branchial sacs joining in a common gut, which then bifurcates into two intestinal buds, which reunite at their distal ends, only to bifurcate again into two recta.

Or, the old pharynx, oesophagus, and rectum degenerate, leaving the new pharynx and rectum in connection with the old intestinal coil. In the first case the compound organism separates into two persons by the splitting of the common pieces of oesophagus and intestine, and this splitting takes place in such a way that the old intestine and rectum remain connected with the new pharynx, whilst the old pharynx enters into connection with the new intestinal coil. In the second case the person is "rejuvenated" by the substitution of a new pharynx and rectum for the old structures.

It will be thus seen that the budding of Urochorda calls up the same kind of problem as does the budding of Polyzoa, but in an even more acute form. In the Polyzoon bud two totally distinct organs, the **circle of lophophoral tentacles,** which are protrusions of the ectoderm, and the **gut** which in the larva is formed from endoderm, arise from the same ectodermal rudiment *in the bud* and are known collectively as the "**polypide.**" This polypide degenerates from time to time, and is replaced by a new one developed in the same way as the old. In the larva of Urochorda the atrial cavity and the nervous system arise from the outer layer, but in the bud they originate from the inner layer. In stolonial budding the inner layer of the bud arises from the parental endoderm, but in pallial budding it arises from the parental ectoderm. Finally, as we have just seen, in Diplosomidae three distinct bud rudiments coalesce to form a new individual, and the part of the individual produced by one of these buds can degenerate and be replaced like the "polypide" of Polyzoa.

How are these facts to be reconciled with the doctrine of germ layers? If the distinction between ectoderm and endoderm be the first and fundamental differentiation of egg substances, how can an ectodermal organ, like the nervous system in Urochorda, be produced from endoderm; or, *vice versa,* how can an endodermal organ, like the gut in Polyzoa, be produced from ectoderm.

Hjört's suggested explanation of this anomaly is extremely plausible. He first of all gets over the difficulty that the inner layer of the bud is formed from endoderm in *Clavelina, Distapha, Perophora,* but from ectoderm in *Botryllus.* The double-walled vesicle, he maintains, is to be regarded as the starting-point of the bud as a new organism, and is equivalent to the ovum, which is the same whether it appears in ectoderm or endoderm. But the outer wall of the bud is in all cases formed from the outer ectoderm of the parent, which is a highly specialized tissue committed irrevocably to producing cellulose, and which is therefore incapable of being modified into nervous tissue. The ectoderm of the embryo, on the contrary, consists of comparatively undifferentiated cells, capable of plastic modification in many directions. The inner layer of the bud, whether derived from epicardium or atrial membrane, consists of cells in a plastic condition, and therefore gives rise to all the organs of the "blastozooid" except the cellulose-producing ectoderm. In the bud of the Polyzoon, if we follow an analogous course of reasoning, the ectodermal outer layer consists of cells in a plastic condition, and all the organs of the bud must arise from it, since endoderm in the bud is conspicuous by its absence, *i.e.* there is no inner layer.

Another way of looking at the matter, however, occurs to one on thinking over the lessons learnt from the development of the egg of *Cynthia*. We have learnt that the cytoplasm of the egg in this form contains specific organ-forming substances; and that the germinal layers and the rudiments of the organs in the developing embryo are distinguished from one another by the possession of different organ-forming substances. We can *see* this in *Cynthia*; the presumption is that such substances distinguish the germinal layers from one another in the case of embryos right throughout the animal kingdom, though the chemical differences between them do not usually reveal themselves to our eyes by different colours. The formation of these substances we have seen to be the work of the nucleus of the developing oocyte, while their final arrangement is due to the spermatozoon, or rather, we might say, to the zygote-nucleus, for the spermatozoon and egg-nucleus come together by a mutual attraction. But, once the definite location of these substances is fixed, it appears as if the nuclei of the segmenting egg were powerless to affect them. Pressure experiments on the eggs of Echinoderms, and even on highly specialized eggs like that of *Nereis*, show that these nuclei can be juggled about like a handful of marbles, and made to change places with each other without affecting the disposition of the organs of the embryo. Thus the specific quality of organs depends on the cytoplasm of the egg, and not on its nuclei.

But it seems certain that this passive condition of the nuclei is only a transitory phase. Leaving aside the obvious fact, that when the generative cells are formed the nucleus must resume its active rôle, there is evidence to show that the specific ferments which characterize certain gland cells, such as the pancreatic cells, are formed out of particles emitted by the nucleus. It is then reasonable to assume that, in the formation of buds, the nuclei again become active and manufacture organ-forming substances in the cytoplasm, but it does not follow that the arrangement of these will be the same as in the egg. It may be that the possibility of the formation of these substances is bound up with a certain quality of the cytoplasm, and the nuclei in the outer layer of the bud may attempt to do this and may fail. We may reflect that in *Ciona*, if any of the cells of the developing embryo be killed, a portion of the tissues of the larva will be missing, and that the larva is unable to make good the defect; and that yet, if the larva be allowed to metamorphose into an Ascidian, and the whole upper part of the young *Ciona*, including the ganglion, be bitten off, the stump can regenerate the missing parts. That being so we can realize the difference between an active and an inactive condition of the nuclei.

It has been hinted above that, since *Ciona* possesses two large thin-walled epicardia, the view has been held that it and the other simple Ascidians once had the power of budding and lost it. The study of the larvae of simple Ascidians does not bear out this view. In the general disposition of their organs, and in the formation of their stigmata, they are more primitive than the simplest compound Ascidian, *Clavelina*. If, then, the epicardia were not originally connected with budding they may possibly represent anterior coelomic sacs—perhaps the collar-cavities since they lie externally to the other viscera.

The origin of the pericardium as a single median evagination of the pharynx is, however, hard to explain. The pericardium of the higher Vertebrata is formed from a portion of the splanchnocoele behind the branchial region, and this splanchnocoele is simply the lower end of the front part of the conjoined trunk-cavities. Now the trunk-cavities of the Urochorda are mainly represented by the longitudinal muscles of the tail, and by the so-called anterior mesenchyme cells which form the mesoderm of the adult, and actually, for some time, form part of the lateral walls of the embryonic gut. Yet there can be little doubt that the pericardium and heart of Urochorda are homologous with those of higher vertebrates; they are situated in the same position in the adult, and the heart, by its shape, reminds one of the S-shaped heart of the higher vertebrate embryo.

Perhaps the only suggestion which can be offered is this. The pericardium is held back in development; it does not develop until the later part of larval life, and its origin from the pharyngeal wall may represent the outgrowth of the trunk-cavities from the archenteric wall of *Amphioxus*. We may suppose that the front portions of these cavities develop late, and independently of the hinder portions, just as we explain the larval and adult mouths of Echinodermata as the two parts of an ancestral mouth, one of which is held back in development. A detailed study of the development of Larvaceae would probably throw light on this question.

In any case there can be no doubt that the ancestor represented by the Ascidian tadpole, and in certain degree by the adult in the group Larvaceae, possessed a well-developed brain-vesicle with hemispherical visual optic lobes, a hypophysis or pituitary body opening into the stomodaeum, also a well-developed tail, and a definite heart; and that in all these respects it was far in advance of the stage represented by *Amphioxus*. The distance which separates the points at which *Amphioxus* and Urochorda diverged from the main stem of Vertebrata, is almost comparable to that which separates the points of origin of *Amphioxus* and Enteropneusta from the same stem.

[*Note.*—It is necessary that this group of animals, lying, as it does, on the borderland of both Invertebrata and Vertebrata, should be treated by the authors of both Vol. I. and Vol. II.; and, as each author is solely responsible for the facts and opinions contained in his book, the reader must be prepared to find that the views adopted by each author, especially regarding this overlapping group, may not wholly coincide.—EDITOR.]

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CHAPTER XVIII

SUMMARY

OUR survey of the embryology of the Invertebrata is now completed, but, before closing the volume, it seems desirable to pause for a brief space and reflect, so that, if there are any general principles to be deduced from the study of the series of life-histories which have been described, they may not escape us.

The first and most far-reaching conclusion which we may draw, is that, in general, the **larval phase** of development represents a former condition of the **adults** of the stock to which it belongs. This, in substance, is of course the **recapitulatory** theory of development, the famous **biogenetic law** of Haeckel. In these days this law is regarded with disfavour by many zoologists, so that to rank oneself as a supporter of it is to be regarded as out-of-date. The newest theory is, however, not necessarily the truest; and this we may certainly say, that if there has been evolutionary change at all, which no one seriously doubts,—if every species of animal has not been created, adapted to the conditions in which we now find it,—then, nothing can be more certain than that the parasitic members of the great natural groups are descended from ancestors which conformed in their structure to the normal type of the group in question.

Now, in the life-histories of these parasites, the larva, in almost every case, shows an unmistakable resemblance to the normal type of adult in the group. It appears to us that we have in such animals a critical case by which we can test the truth of the recapitulatory theory; since, so far as human intelligence goes, the ancestry of such creatures is known. Does any naturalist seriously doubt that the ancestors of *Actheres ambloplitis,* described in Chapter VIII., were once ordinary Copepoda? As Metschnikoff has said, parasites are really the latest products of evolution.

If, however, the recapitulation of ancestral structure turns out to be the primary explanation of developmental history in cases where the ancestry is known, surely we have the right to assume that the same type of explanation is valid where the ancestry is otherwise unknown, and to conclude that in general the larval phase has a recapitulatory significance.

Of course this conclusion was tacitly assumed by all evolutionary zoologists thirty years ago, but few have paused to think what consequences are implied in this conclusion. Among these few must be reckoned Sedgwick (1909), who points out that on this theory it follows that, as time goes on, the life-cycle must be ever tending to grow more complex, since new phases are always being added to it at its adult end. Another way of phrasing this conclusion is to say that a new step in evolution usually takes place when the adults of a species seek a new environment, and in reaction with it have their structure modified.

If we inquire whether the adults of a species do often seek a new environment, then some very interesting evidence may be adduced. Allen (1899), as a result of several years' painstaking exploration of that portion of the English Channel lying in the neighbourhood of Plymouth Sound, has shown that each marine species has a particular type of bottom which is suited to it, and which may be termed its **home**. On ground of this kind it swarms; but around the areas of this type of bottom numerous stragglers of the species are to be found. It seems clear that, from the home population, crowds of colonists are for ever being sent forth which, in most cases, fail to maintain themselves, but which may in rare cases successfully establish themselves, and in this way a new race or species may be produced. Every species, indeed, can be compared to a fire in the midst of a dry prairie, which for ever tends to extend its borders.

Now when an animal encounters a new environment one of two things will result; either its metabolism, and as a consequence all its activities, will be checked; or the metabolism will be promoted and the vigour of its life increased. In the first case it will either die or lead a stunted and sickly existence; in the second case its structure will almost certainly be modified. This modification may be described as a reaction to the stimulus of the new environment; and as environment can be analysed into a few factors, such as food, temperature, moisture, salinity, etc., the modification must, in the last resort, be the effect of one or more of these on the metabolism. When, therefore, we find that the larva, as compared with the adult ancestral stage which it represents, is almost always very much reduced in size, and that the change to the adult condition takes place whilst the animal is still very small, one receives the impression that one is dealing with a reaction which, constantly repeated through thousands, nay, myriads of generations, tends to set in sooner and sooner in the course of the development; just as in the life of the individual, *the formation of habit* causes reactions to require for their evocation less and less of the original stimulus.

But, the reader will exclaim with horror, does not this explanation postulate the acceptance of that Lamarckian heresy, the inheritance of acquired characters? and have not experiments shown such an idea to be devoid of foundation? The answer to this question is twofold: first, the difficulty of framing any other theory of recapitulation seems to be insuperable; and, second, the experiments which have been held to disprove the inheritance of acquired characters are far from conclusive.

To take the first point first: If the chequered course of developmental history is the result of the preservation of chance variations in the struggle for existence, then we have to assume that, in the eggs of animals living in one environment, variations occurred which were suitable to new environments, and which manifested themselves at the period of adolescence. That, to take a concrete example, among the eggs of the Copepod ancestor of *Actheres*, some varied so that at adolescence the organisms developed from them tended to lose all their appendages, and that this loss had nothing to do with the reaction to the new environment (gills of fish) in which the animals found themselves. Or, to take another instance, the hermit crab did not acquire its curved abdomen in consequence of the habit of thrusting it into the empty shells of gastropod molluscs; but since the curvature appears in these animals when they are reared in confinement from the larval stage and prevented from finding shells at all (Thomson, 1904), we must assume that in this species the tail became curved in the proper spiral by chance variations, and that then its possessor formed the useful habit of seeking gastropod shells to clothe it. Such explanations are perhaps not impossible, but, to speak frankly, they do not commend themselves to us.

To consider now the second objection. The evidence from experiments is not at all conclusive. The earlier experiments designed to test the inheritability of acquired characters, can only be described as childish. They consisted in such things as cutting off the tails of mice, and in rearing the offspring of these mutilated animals, in the expectation that the young would be born without tails. If anything acquired can be inherited it must surely be some reaction of the organism as a whole. Now Kammerer (1913) has shown that, by keeping salamanders for several generations in certain environments marked changes in skin coloration result; and that, after a time, not only do the beginnings of these changes show themselves in the young before they are exposed to the new environment, but these altered forms, when crossed with the original forms, behave like Mendelian races (see Chapter I.).

It must be remembered that the experiments of the Mendelian school of biologists, which are held to demonstrate the unalterable character of the reproductive substance or germ-plasm, have been continued through only a very few generations; whilst to obtain the inheritance of acquired characters the action of the new environment would probably, in most cases, be continued through thousands of generations. The evidence from Palaeontology seems to suggest that, in making structural advances, Nature acts with extreme slowness. It required the whole length of the Secondary Period to make a crab out of a lobster-like ancestor, and the whole of the Tertiary Period to convert a five-toed mammal into a horse.[1]

The dislike with which the Lamarckian theory is viewed is not entirely due to the supposed evidence against it furnished by experiments. Two other considerations have been urged: first, that there are modifications which cannot have been the result of the inheritance of acquired characters; and, second, that it is difficult to conceive of any mechanism by which the characters of the body can be transferred to the germ.

In the first objection there lurks some confusion of thought. It is tacitly assumed that acquired characters must be changes of structure due to new habits acquired by the animal in *adaptation* to its new environment. But to assume this is to narrow, in an unwarrantable fashion, our conception of what constitutes an acquired character. The reaction to the environment need not be an adaptation. Certainly' it will only be preserved if it happens to be an adaptation, or if it is at least not harmful. But even with this widening we must admit the justice of Lankester's criticism of such explanations, as applied to the habits of insects in feigning death: "An insect either postures and escapes, or it does not posture and it is eaten; it is not half-eaten and left to benefit from experience."

But our aim is not to explain the origin of all specific differences —it is the much more modest one of attempting to give a rational account of recapitulation.

As to the second consideration which is urged against the inheritance of acquired characters, viz. the difference of forming a conception of its *modus operandi*; its great weight must be admitted, but even here we are beginning to receive light from both physiology and the new science of experimental embryology. The discovery of **hormones**, by Starling, is of far-reaching importance in this respect. These are substances, produced by certain organs of the body and poured into the body-fluids, which have a powerful effect on growth and other metabolic processes. Now, it is quite possible and even probable that the few hormones which so far have been discovered, such as the hormones of the thyroid and pituitary glands, have been recognized on account of their exceptionally active chemical character, and that hormones of lesser strength are given off by all the tissues of the body, and that the various organs of the body stand in a sort of mobile chemical equilibrium with one another.

Considerable support can be drawn from experimental embryology in support of this view. Herbst (1899) has shown that if the eye of the shrimp be cut out, a new eye will be regenerated so long as the optic ganglion is left intact, but that if the optic ganglion be cut out, an antenna-like organ will be regenerated instead. The only possible explanation of this fact is to conclude that some influence—a hormone, in fact—emanates from the optic ganglion, which so influences the regenerating ectoderm as to determine that it shall take on the extremely complicated structure of the compound eye.

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[1] Since these lines were written an interesting example of the stereotyping of a functional reaction has occurred to us. In the lagoons of Prince Edward Island, where the oyster swarms, two varieties occur: one, the so-called Cup-oyster, on hard shelly or gravelly ground; the other, the Mud-oyster, on mud. In the latter the edges of both valves are curved up so as to keep the opening above the mud; in the other the edges of the valves remain horizontal. Both varieties develop from the same larvae. Now in secondary strata there occurs a genus, *Gryphaea*, which is just a Mud-oyster stereotyped, and it is found in clay deposits.

A still more striking case has been described by us (MacBride, 1911). In the development of the ordinary sea-urchin normally only one water-vascular rudiment or hydrocoele is formed, and this is situated on the left side of the larva. The ectoderm lying above it becomes invaginated to form a deep sac—the amniotic sac. From the floor of this sac are developed the adult nerve-ring and adult spines. In the centre of the floor an invagination is formed which gives rise to the adult mouth. From the peritoneal epithelium of the left coelomic sac, which lies beneath the hydrocoele, are developed the dental pockets and the teeth which project into them.

But occasionally a second hydrocoele is developed which is situated on the right side of the larva. When this takes place the ectoderm on the *right side* of the body becomes invaginated to form a deep sac, from the floor of which a second nerve ring, a second set of adult spines, and a second mouth are developed, whilst the underlying peritoneal epithelium of the *right coelomic sac* gives rise to a second set of dental pockets.

Now, although we have good reason to believe that the common free-swimming ancestor of all Echinoderms possessed two hydrocoeles, yet it is as certain as anything can be that this ancestor did not possess two mouths surrounded by nerve rings, nor did it have the highly differentiated spines and teeth of the Echinoid. The production of a second hydrocoele is probably an ancestral reminiscence, but the production of these other organs cannot possibly be so explained.

The only conclusion, therefore, that we can draw is that, in the natural development of *Echinus,* the hydrocoele emits a hormone which causes the ectoderm to form the amniotic invagination, and which causes the left coelomic vesicle to give rise to the dental pockets. It follows further, that particular portions of ectoderm and of coelomic wall are not specialized so that they alone can undergo these modifications, but that any portion of the ectoderm and any portion of the coelomic wall can undergo similar modifications, provided that they are acted on by the appropriate hormone, as is shown by the effect of the right hydrocoele on the right coelomic sac and on the ectoderm of the right side. Development then is a kind of progressive specialization, due to the influence of one organ on another by means of hormones.

Professor Langley has pointed out to us (*in literis*) that if an animal changes its structure in response to a changed environment the hormones produced by the altered organs will be changed. The altered hormones will circulate in the blood and bathe the growing and maturing genital cells. Sooner or later we may fairly assume that some of these hormones may become incorporated in the nuclear matter of the genital cells; and then, when these cells develop into embryos, the hormones are set free at the corresponding period of development to that at which they were originally formed. They reinforce the action of the environment and cause it to produce greater effects; they may become free even before the stimulus of the environment reaches them, and produce the appropriate structural change at an earlier period of development. In this way we explain the tendency not only to recapitulate, but to reflect back ancestral structures into progressively earlier periods of development.

We do not assume for a moment that this is a full and satisfactory explanation of recapitulation, we regard it merely as a sketch of the direction in which the explanation may be found, and as a call for further investigation.

But recapitulation of ancestral structure is by no means the only factor in development; and we must now inquire whether our studies have led us to form an idea of what the other factors are. One has been already alluded to, viz. the tendency for changes in structure to make their appearance in successively earlier periods of growth, and consequently for the larva to be reduced in size. That this reduction in size entails changes in the larval organs has already been pointed out. We have seen that serially repeated organs, like the legs of the Nauplius, may be reduced to two or three pairs of those which are functionally the most important; that organs which should occur in pairs, arranged in a bilaterally symmetrical manner, may be represented by one-sided structures, such as the eye of the Ascidian tadpole and the "primary" gill-slits of *Amphioxus*.

Even here the researches of experimental embryologists may aid us. Driesch (1900, 1910) has succeeded in inducing two or three early larvae of *Echinus* to fuse into a single compound organism. This compound larva naturally possessed three guts, but one only grew large and became functional, the others dwindled, no doubt owing to some inhibiting influence on their growth emitted by the larger one. So we may imagine that larval organs which are functionally necessary, and which increase, owing to their use, out of all proportion to the proportion they should sustain to the organism in which they find themselves, must inhibit the growth of their less fortunate sisters.

Again, we must remember that whilst the reaction to the final environment has evoked and sustains the adult structure, the larva has also its environment. Whilst in many, nay most cases, we have reason to believe that the larval environment has the same general characters as the environment of the ancestral stage which the larva represents, yet in no case is it probable that the two environments are exactly alike, and in some cases the larval environment has become markedly different from what the ancestral one must have been.

Even the reduced size of the larva and the tendency for adult organs to develop precociously will alter its relation to the environment. The enormously long ciliated arms of the Ophiopluteus larva are necessary to sustain the growing weight of adult calcareous plates which make their appearance in the later stages of larval life. The free-swimming ancestor represented by the larva had almost certainly no calcareous plates at all.

When, as in the case of insects among Arthropoda, and Unionidae amongst Mollusca, the larvae take to special modes of life, the modifications of the recapitulatory history become profound, and special larval organs are produced; such as the tracheal gills of the nymphs of Ephemeridae, or the hooked apices of the shell-valves in the Glochidium larva, which never existed as organs in any adult ancestor, and whose presence may be regarded as a falsification of embryonic history.

If it be asked how these secondary characters are to be discriminated from primary larval characters, the answer is that *primary larval characters connect different groups of the animal kingdom,* such as the organs of the Trochophore found in almost identical form in Mollusca, Annelida, and Polyzoa; or the Nauplius larva found in Phyllopoda, Copepoda, Cirripedia, and Malacostraca. *Secondary larval characters are confined to smaller groups,* and sometimes betray their secondary character by their peculiar structure. Thus the tracheal gills, alluded to above, allow the oxygen dissolved in the circumambient water to come into proximity, not with blood contained in these gills as in all other gills, but with air contained in them, from which in turn the blood derives its oxygen. Here is the clearest indication that in the ancestral stock the larva was originally air-breathing in habit and took to water as a shelter.

But we have learnt to recognize yet another modifying factor in development. As the race progresses in evolution and successively seeks new environments, there will be a tendency to leave behind it a trail of larval stages representing past conditions of the stock. In some cases, as in the life-history of *Penaeus,* at least four successive larval stages can be recognized. But in each larval stage the species is exposed to special dangers and suffers enormous mortality. If therefore the earlier larval stages can be passed through either within the mother's body or inside an egg-shell, this mortality will be greatly lessened.

This change has taken place to some extent in all life-histories, for every life-history starts with an **embryonic phase.** In some life-histories almost the whole of the development takes place under shelter, and the young animal steps out into the world ready to take up the adult mode of life. Such life-histories are said to be of the embryonic type. They are so advantageous from the point of view of infant mortality that the wonder is that any life-histories remain in which the larval phase is predominant.

We must believe that the advantage to the race which the wide dispersion, resulting from the existence of a free-swimming larval phase, confers on it, counterbalances the disadvantage resulting from the mortality of the larvae. Generally speaking, the more modified and more advanced in structure a species is, the more prominent is the embryonic phase in its life-history. But in isolated cases, within each natural group of animals, almost the whole of the life-history may become embryonic, whilst the adult structure remains comparatively unmodified. So we find that in *Peripatus capensis*, one of the most primitive of Arthropods, the whole of the development is passed within the maternal oviduct; and that in *Amphiura squamata* the young emerges from the genital bursa of the mother as a perfect brittle-star, whilst nearly allied species have a long larval development. Such cases could be multiplied, and there can usually be found some reason in the peculiar local conditions of the species which makes a larval life peculiarly dangerous.

But even when larval life has begun, its even course is sometimes interrupted by phases which we may term **pupal**, and which show a striking similarity to embryonic phases. During these the growing form may shelter itself in some kind of case, either of extraneous matter or of its own secretions, and takes no food, whilst extensive internal changes go on. In less marked pupal stages the creature continues to lead a free life but takes no food. We may recall to the reader's mind the pupal stages of Cirripedes and Holothuroids.

The reason for these modifications is clear. In every larval stage, as we have seen, the creature encounters dangers and has a special way of obtaining food. In one and the same locality these different ways are probably not equally effective. It is therefore a great advantage if enough food can be accumulated during one larval stage to enable the next to be passed rapidly through without needing food at all. The most common form of this is to find that the larvae, when just hatched, retains a store of food in its tissues from the preceding embryonic stage, and whilst it moves about freely it requires no food. The yolky larvae of *Asterina, Cribrella*, and *Solaster*, among brittle-stars, may be mentioned, as also of most Ectoproct Polyzoa. Such creatures are really intermediate in type between embryos and larvae.

Finally, in rare cases, the alteration of conditions, either of climate or of the nature of competitors, may cause the larval stage to become more safe and advantageous than the adult; and in this case the adult stage is employed only for pairing and egg-laying, and is passed quickly through, or it may be suppressed altogether. Originally the conditions of adult life must have been more advantageous than those of the larva, or the evolutionary step from the one to the other would never have been taken. But we have already pointed out that the imaginal stage in many of the higher insects is of very short duration, and that during this stage, in many cases, no food is taken; and here we must assume that the life of the larva is less dangerous than that of the adult. Again, among some Urodeles, such as the Axolotl, the larva can develop ripe genital organs whilst continuing the larval mode of life, and then the adult stage, for the evocation of which the appropriate stimulus has ceased, drops out. As is well known, this lost adult stage can be evoked in the Axolotl when the proper stimulus is discovered and artificially applied.

From considerations of the secondary factors which modify larval life, we pass to the factors which modify embryonic life. Of these the most important is food. Food is supplied to the embryo either in the form of yolk grains embedded in the cytoplasm of the egg, or of maternal secretions. These two forms of food give rise to different kinds of modifications. To take the yolk first. Its presence in any quantity slows down cell division, as Balfour (1881) has pointed out, and in extreme cases abolishes it altogether, leaving only division of nuclei. It causes the number of cells produced to be smaller, and the size of the individual cell to be larger, and so it renders such processes as the formation of pouches, or of folding, impossible, and they are replaced in the yolky embryo by processes of solid proliferation of cells. Ordinarily speaking, yolk is stored in the cells which will afterwards form part of the gut, but in certain cases, as in certain Echinoderm embryos, it is more widely distributed; and then the most profound modification in early development can take place, the whole of the archenteric wall may be directly converted into coelom, and the true gut formed later as an outgrowth from this.

Maternal secretions are in most cases absorbed through the ectoderm, and the effects of this change of function on this layer are profound. The extraordinary spongy ectoderm of *Peripatus capensis* is due to this, but the most striking instances of the effects of this kind of food on the development of the embryo are found amongst Mammalia, and will doubtless be dealt with in the third volume of this work.

Finally, it must be remembered that, in the embryonic phase of development, the functional correlation of the development of the various organs necessary to the *larva*, as to every free-living self-supporting organism, can be profoundly altered without impairing the end of development—viz. the successful attainment of the adult form. The structure of the embryo, at any period, is the outward and visible expression of the co-working of independent processes, held together by a very loose rein. A beautiful instance of this has been given by Jenkinson (1906). In the embryo of Urodela the lower part of the blastopore remains continuously open and eventually forms the anus. In the Anuran embryo this part of the blastopore closes, but the anus is formed later as a new perforation in identically the same place. Now, by allowing the Anuran egg to develop in solutions of certain salts, the blastopore can be caused to remain open and the egg then develops after the Urodele manner. The question as to whether the blastopore persists or not is, therefore, merely a question of the value of the differential equation connecting the rates of growth of the archenteric walls, and the rate of expansion of the archenteric cavity.

Speaking of the geological record, Darwin wrote, in 1859, that of the book of life we only possess the last volume. This conclusion, so far as Invertebrates are concerned, has been amply confirmed by the remarkable discovery by Walcott (1912), of a richly differentiated and exquisitely preserved fauna in the Mid-Cambrian rocks of North America. If therefore the relationship to one another, not only of the various phyla of the animal kingdom, but even of the classes within the phyla, is ever to be elucidated, it can only be done by Comparative Embryology. Palaeontology begins too late to undertake the task.

From all the discussion, however, which has just been completed, of the secondary factors which modify the recapitulatory record in both embryonic and larval life, it will be clear that only an Embryology based on an enormous collection of facts, and a careful comparison of one type with another, can hope to discriminate between primary and secondary factors in development, and to elucidate ancestral history.

It is because too many zoologists have based far-reaching theories on the life-history of some one type, that recapitulatory embryology has fallen into bad repute. But the remedy for the abuse of little light is more light, and already, by the steady accumulation of embryological data and the improvement of methods, some of the controversies which vexed our fathers in the 'eighties are in a fair way to be settled.

The late Professor Welldon pointed out that it would be of great interest and importance to find out how far the most recent evolutionary changes, such as those which made the difference between genera within a family, and between families within a tribe, are represented in the life-history. Here is a field for research of great importance, in which very little work has as yet been done. These latest stages of the life-history should be the freest from disturbance by secondary factors, but it is precisely over the interpretation of the earliest stages in the life-history, which represent the oldest pages in the ancestral record, and which one would expect to be most blurred, that the greatest disputes have arisen.

There is a school of embryologists, led by Driesch, who decry the value of the recapitulatory interpretation altogether, and who insist that the developmental processes of every animal should be referred back to causes existing in its egg, and that no hypothetical ancestry should be called in to aid in the explanation. No doubt it is true, theoretically, that the stages in the development of an animal form a continuous series, and that the causes for the production of each stage are to be found in the preceding one. But even if this causal chain were completely elucidated, it would leave entirely unexplained the marvellous resemblances between the larvae of some species and the adult stages of others, or between the larvae of widely different groups; and it is precisely phenomena of this kind that Comparative Embryology seeks to account for.

In this search Comparative Embryology has been very greatly aided by the science of Experimental Embryology, founded by Roux, and so ardently followed by Driesch, Herbst, and many others. If we ask ourselves what is the most remarkable achievement of this science, the answer must surely be the discovery of **organ-forming substances.** We have already pointed out that such substances are emitted by the nucleus of the ripening egg into the cytoplasm, and that they confer on the latter a definite character; and that the early course of development consists in separating these substances from one another, and in allocating them to different regions of the segmenting egg and embryo. This separation can occur at an earlier period in one type of egg (Annelida, Mollusca, Ctenophora, Tunicata), and at a much later period in another type of egg (Hydrozoa, Echinodermata). Until it has been effected a portion of the egg will produce a perfect embryo of reduced size.

We now see the inner meaning of that much debated "formation of the layers," over which so many battles have been fought. Pressure experiments prove abundantly that, after the primary emission of organ-forming substances, the nuclei of the developing embryo form an indifferent material, and what settles the fate of any cell is the quality of the organ-forming substance locked up in it. But, as we have already pointed out, this indifference of the nuclei is a passing phase, because, for one thing, if it were permanent, the male pronucleus brought in by the spermatozoon would have no effect in determining development, which is notoriously not the case. The later course of development may be regarded as a result of the action of already-formed organs on one another by the aid of hormones. But indeed the organ-forming substances themselves are most plausibly regarded as of the nature of hormones or ferments. This is clear from the consideration that the quantity of the organ-forming substance can vary within wide limits and yet give rise to a perfect organ. A certain minimal quantity in the fragment of an egg will lead to precisely the same result as a much larger quantity (cf. Boveri's experiments on the eggs of *Ascaris*, Chap. XV.).

We cannot for a moment imagine that the molecules of the organ-forming substance are little pictures of the organ which it is their function to produce. Of course it is a mere commonplace to say that we are only on the threshold of our knowledge of these substances, and are still very far from understanding their *modus operandi.* How is it, for instance, that a slight prick with a knife in the bud from which a new tail is regenerated in a lizard which has lost its tail, is capable of bringing it about that two tails, and not one, are developed? So far as our knowledge goes, it seems to be clear that for the formation of an organ of what we may call the second order (*i.e.* such a thing as a head or a limb), as distinguished from an organ of the first order, or a germinal layer, the co-operation of at least *two organ-forming substances, in definite spatial relations to each other*, is necessary.

If the spatial relations be altered we may get *two* organs formed by the co-operation of the substances, instead of one. If, for instance, the 2-cell stage of a developing frog's egg be compressed between two glass slides so that it cannot rotate as a whole, and the preparation inverted, a double-headed tadpole will result. Here nothing has been removed, nor have the connections of the blastomeres with each other been destroyed, but the spatial relations of the organ-forming substances have been altered under the influence of gravity, and as a result the tadpole has two heads instead of one. Of a quite similar nature are the results obtained by Spemann (1901, 1903) on the gastrula of the newt, when he employed constriction by a string, and a two-headed monster resulted. So much we can determine empirically, but the how and why are still to seek.

The reader will see that the door is opened to a host of interesting questions, and that indeed experimental embryology passes into cytology, for the whole question of the relation of the nucleus to the cytoplasm is raised. To take one example of such questions, we may ask, if the nuclei of the segmenting egg are indifferent material, and the distribution of cytoplasmic organ-forming substances is fixed at the moment of fertilization, and if these substances, as seems certain, are of purely maternal origin, at what point in development does the male influence assert itself? For this point must coincide with the point of renewed nuclear activity. A series of carefully chosen hybridization experiments should throw light on this problem.

Again, we have seen reason to ascribe the formation of buds to the reassumption of an active rôle on the part of the nuclei of adult tissue, and the renewed formation of organ-forming substances. We have ascribed the different course of the development of buds from that obtaining in embryonic development to a different distribution of these substances. Can we detect microscopically any difference between these active nuclei and the normal inactive nuclei? Here again is a fruitful field for research.

Let us in the meantime, adopting the recapitulatory view, provisionally sketch the developmental history of invertebrates so far as our present knowledge extends.

In the larval history of the least modified Sponges, Coelenterata, and Echinodermata—groups which, in the differentiation of their adult organs, are the lowliest amongst Invertebrates—the first distinct larval phase is the **blastula,** which is a hollow sphere or ellipsoid, whose walls are constituted by a single layer of flagellated cells. Since this larval phase is represented in a form more or less obscured by secondary changes in the embryonic life-history of all the other groups, we may take it as representing in rudest outline the form of the common ancestor of all Metazoa. It resembles in many respects certain existing colonial Protista, such as *Volvox,* sometimes claimed by zoologists and sometimes by botanists.

Such a simple, free-swimming stock must have swarmed in the shallow, warm seas of the primitive globe, when there were no higher forms to compete with it. At first every cell fed itself like every other, but later, when some members of this widespread stock took to a creeping life, then the lower cells, in contact with the nutritive substratum, remained nutritive, but the upper cells became protective, and formed a covering dermal layer; and so the group of Sponges or Porifera were evolved.

In those members of the stock which remained free-swimming, however, the direction of progression changed from an indefinite rotation to a definitely directed progress; the hinder cells became adapted to catch nutriment, the anterior became purely sensory. The nutritive cells became increased in number and invaginated, and so the primary gut was formed, and the stock were no longer blastulae but **gastrulae.**

The **gastrula** stage exists as a larval phase in more cases than does the blastula one, and it is likewise represented, in obscured form, in the embryonic history of the higher types. Some of the gastrula stock likewise took to a bottom life, and gave rise to the Hydrozoa, Scyphozoa, and Actinozoa, amongst Coelenterata.

The main portion of the stock remained, however, free-swimming, and developed lateral pouches of the gut, in which excretory and reproductive functions were specialized, and which also gave rise to cells of a specially locomotor character; while at the aboral pole the sensitive cells had become a definite nerve centre.

In this way a primitive wide-ranging group was evolved, supplanting the gastrula stock, to which we may give the term **Protocoelomata.** In much modified and specialized form this stock survives as the Ctenophora at the present day. But the Protocoelomate stage in the development of the race is represented in the ontogeny of Invertebrata by three distinct types of larvae, viz. the Trochophore larva, the Echinoderm larva, and the Tornaria larva. The latter two types of larva probably do represent the same type of ancestor, but the Trochophore larva, with its early specialized development, is different; it is more closely allied to existing Ctenophora, and the coexistence of these two types points to the existence of different types of specialization amongst the original Protocoelomate stock. The Brachiopod larva is in many ways intermediate in character between the two types.

From the division of Protocoelomata represented by the Trochophore, bottom-living forms were produced. These, as we have pointed out in the proper place, are the burrowing Annelida, the creeping and gliding Mollusca, as well as Podaxonia, and Ectoproct and Entoproct Polyzoa. The Nemertinea are also bottom-haunting forms descended from the same stock. The Platyhelminthes represent an earlier offshoot from the Trochophore stock, before the primitive mouth had been cut into definite mouth and anus, and the lateral branches of the gut definitely specialized as coelom. The Brachiopoda are a bottom-living group, descended from a type of Protocoelomata intermediate between the Ctenophore-like ancestor presented by the Trochophore larva and the Dipleurula ancestor of Echinodermata.

From the Annelida arose Arthropoda, whose members in some degree regained the free-swimming life of their far-off ancestors; but they never regained domination in the open sea, for, in the meantime, that portion of the Protocoelomate stock represented by the Dipleurula had advanced in development, developing long ciliated tentacles for the prehension of food; and this section of the stock leads straight on to the victorious Vertebrata, whose leading members have never deserted the free life of the ocean rover, and have been and are now dominant in the seas.

From time to time weaker brethren have given up the struggle and sought safety in a bottom life; of these the oldest offshoot are the Echinodermata, later came the Cephalodiscida, and then the Balanoglossida, which, though classed together as Enteropneusta, represent two separate offshoots from the free-swimming Protocoelomata. Then, much later, the Cephalochorda diverged, later still the Urochorda (Tunicata), and finally the Cyclostome Fishes.

It is, therefore, broadly speaking, true that Invertebrates collectively represent those branches of the Vertebrate stock which, at various times, have deserted their high vocation and fallen to lowlier habits of life.

When once a stock has so fallen, is there a place for repentance? Can a dominant position be regained? At first sight the history of Arthropoda appears to answer this question in the affirmative, for they have certainly progressed far beyond their Annelidan ancestors. As all know, vigorous attempts have been made to prove that Vertebrata are evolved from Arthropoda. The only evidence for this is the superficial similarity between certain early Arachnida and fossil Fishes, coupled with the assertion that Arthropoda were dominant in the seas before Vertebrata, and that a dominant group like the Vertebrata must have arisen from a pre-existing dominant group.

Now the difficulty in the way of this view, even supposing that the enormous differences between vertebrate and arachnid anatomy could be brushed aside, is that the fish which are compared to Arachnida are degenerate bottom-living forms. They are not the earliest and most primitive fish, whose blade-like bodies swept like arrows through the waters above. The Arachnida, with which they are compared, are also almost certainly bottom-living forms, and there is no evidence that the Arthropoda, except in the case of minute-bodied inconspicuous forms, such as Copepoda and Cladocera, ever really took to active life in the open sea again. The larger and heavier types are all bottom-living, and it seems perfectly clear that, when the Arthropoda sought to recover their lost birthright, they found the ground preoccupied by the Vertebrata and their opportunity gone for ever.

In adaptation to life on land, however, Arthropoda were beforehand with their rivals, and in pre-Carboniferous days must have expanded and flourished enormously; but the start was speedily overhauled when, in Carboniferous days, the Vertebrata followed them on to land and, though as Insecta the Arthropoda still maintain themselves and limit the activities of land Vertebrata in many ways, yet if we have regard to bulk of body, and above all to psychic development, there can be no question as to which is really the victorious race.

Our task is finished; we have striven to represent the science of Comparative Embryology, not as a well-ordered and complete system of philosophy, but rather as a gold-mine from which rich rewards have already been reaped, but of which only a small part has as yet been developed, and which promises abundance of gratifying surprises and rewarding returns to the worker of the future.

" *The harvest truly is plenteous, but the labourers are few.*"

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